THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 119 JULY 1, 1937 No. 3

KINETICS OF THE EXCRETION AND UTILIZATION OF XYLOSE

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Received for publication September 24, 1936

The fact that not all the quantity of xylose injected in the body is excreted by the kidney has been known for a long time (see old literature in Neuberg, 1904), but of the fate of the non-excreted portion little is known. In this study a process of "utilization" will be assumed in order to account for the whole quantity injected.

After equilibrium between plasma and tissues, the rate of utilization must be proportional to the plasma concentration (Dominguez, 1934a), but since equilibrium may take some time to be attained, and there is no reason to expect the process of utilization to be different in different stages of equilibration, it is not clear whether the rate of utilization should be proportional to the tissue concentration or proportional to the plasma concentration from the beginning of the experiment. Before these considerations are introduced into the theory of elimination given by us (Dominguez, Goldblatt and Pomerene, 1935[3]), this theory has to be modified in order to remove an undesirable feature of it, first pointed out to one of us by Dr. A. C. Young, of the Department of Physiology, University of Rochester. In figure 1 of the creatinine paper the (hypothetical) tissue concentration is seen to lower before it has reached the plasma level, and since creatinine is not removed by the kidney directly from the tissues, it would appear as if it is transferred from the tissues into the plasma against the greater con-

[1] Aided by the Florence H. Kridler Fund (grant to D).
[2] Aided by the Beaumont-Richman-Kohn Fund (grant to G).
[3] This paper will be referred to as the creatinine paper.

centration of the latter, which is evidently untenable. In order to overcome the objection, a change in the assumption of the creatinine paper is necessary, and we shall adopt the one suggested by Doctor Young. We wish to acknowledge our indebtedness to Doctor Young for his friendly and constructive criticism.

Theoretical part. Calling ξ the concentration of xylose in the plasma, z the concentration in the tissues, η the rate of excretion, u the rate of utilization, P the plasma volume and T the tissue volume, we have

$$-P\frac{d\xi}{dt} = \eta + u + T\frac{dz}{dt}. \tag{1}$$

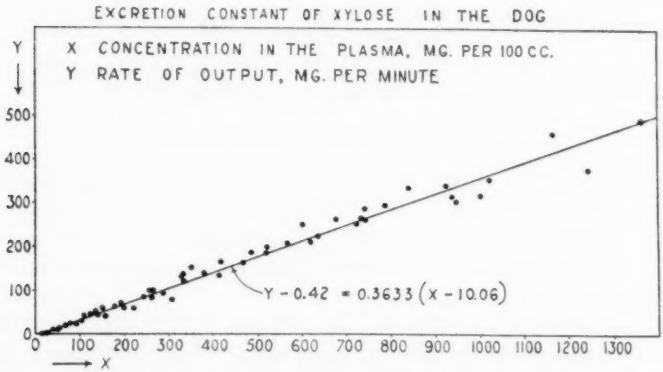
EXCRETION CONSTANT OF XYLOSE IN THE DOG
X CONCENTRATION IN THE PLASMA, MG. PER 100 CC.
Y RATE OF OUTPUT, MG. PER MINUTE
Y
X
500
400
300
200
100
0
0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300
Y - 0.42 = 0.3633 (X - 10.06)

Fig. 1. Relation between the average output of reducing substances in various intervals, y, and the concentration of non-fermentable reducing substances in the plasma, x, interpolated graphically at the middle of the intervals of urine collection. The line joins the centroid of the system (158.97, 446.42) to the endogenous point (0.42, 10.06). The slope of the line, 0.3633, represents the excretion constant of xylose A. Eighty-four points were originally drawn, but not all are visible on account of overlapping near the origin.

In our dogs the rate of excretion of xylose is on an average proportional to the plasma concentration (see fig. 1), as in man (Dominguez and Pomerene, 1934), that is, $\eta = A\xi$, A being the excretion constant. The new assumption concerning diffusion is that the rate of diffusion from the plasma into the tissues is proportional to the concentration difference,

$$T\frac{dz}{dt} = \gamma(\xi - z), \tag{2}$$

γ a constant.

Introducing these results in equation 1 and writing $u = B\xi$, we get

$$-P\frac{d\xi}{dt} = (A + B + \gamma)\,\xi - \gamma z. \tag{3}$$

In this equation, B represents the utilization constant of xylose. It has the dimensions $[l^3/t]$, as A and γ.

The system of equations 2 and 3 has the solutions

$$\xi = ae^{-\alpha t} + be^{-\beta t}, \tag{4}$$

$$z = c(e^{-\alpha t} - e^{-\beta t}), \tag{5}$$

with the conditions

$$b/a = k = \frac{T\beta - \gamma}{\gamma - T\alpha}, \tag{6}$$

$$\alpha\beta = \frac{\gamma(A + B)}{TP} \tag{7}$$

and

$$\alpha + \beta = \frac{(T + P)\gamma + T(A + B)}{TP}, \tag{8}$$

from which we can get

$$c = \frac{\beta + k\alpha}{\beta - \alpha} \cdot a, \tag{9}$$

$$P = \frac{(A + B)(\beta + k\alpha)}{\alpha\beta(1 + k)}, \tag{10}$$

and

$$T = \frac{k(A + B)(\beta - \alpha)^2}{\alpha\beta(\beta + k\alpha)(1 + k)}. \tag{11}$$

In order to calculate B, we have, writing G for the quantity of xylose injected,

$$G = \int \eta \cdot dt + \int u \cdot dt, \tag{12}$$

and after substitution, integration and rearrangement,

$$B = \frac{G - A(a/\alpha + b/\beta)}{(a/\alpha + b/\beta)}. \tag{13}$$

Since the initial plasma concentration ξ_o is equal to $(a + b)$, it is also true that

$$P = G/\xi_o \tag{14}$$

which affords a good check on the numerical work (see equation 10).

Equation 14 shows that the initial concentration ξ_o is proportional to the quantity injected, G, a fact that we have examined directly (see fig. 2).

We have also verified that a too is proportional to G (see fig. 2). It follows that $k = b/a$ is an essential constant, independent of the quantity injected.

If we use the other assumption concerning the utilization of xylose, and write $u = B'z$, equation 3 becomes

$$-P\frac{d\xi}{dt} = (A + \gamma)\xi + (B' - \gamma)z, \tag{15}$$

Fig. 2

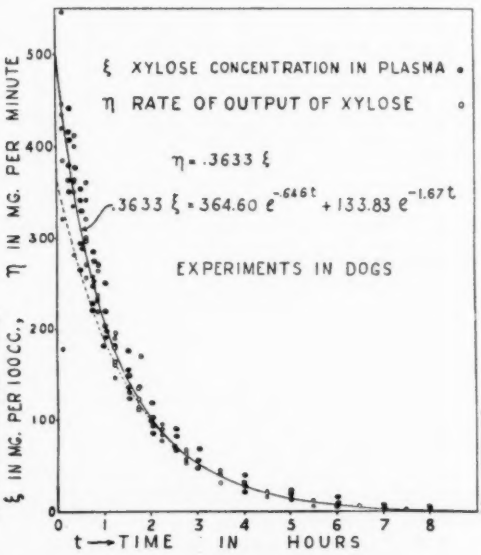
ξ XYLOSE CONCENTRATION IN PLASMA
η RATE OF OUTPUT OF XYLOSE
η = .3633 ξ
.3633 ξ = 364.60 e^-.646t + 133.83 e^-1.67t
EXPERIMENTS IN DOGS
η IN MG. PER MINUTE
ξ IN MG. PER 100 CC.,
t → TIME IN HOURS

Fig. 3

Fig. 2. Relation between the calculated initial concentration of xylose in the plasma ($a + b$) and the quantity of xylose injected rapidly into a vein (G). The relation between the constant a and G is also shown. Note that the large scattering of the ($a + b$, G) points is due to b.

Fig. 3. Observations of the plasma concentration ξ and the rate of output η of xylose, following rapid intravenous injection, plotted against the time. All the observations, 181 in number, have been reduced, as indicated in the text, to a single set of points, from which a mean curve was calculated (solid line of the figure). On account of overlapping several points in the last five hours of the experiments are not visible. Note the erratic observations of the rate of output in the first fifteen minutes. The broken line represents the first exponential only ($364.60e^{-0.646t}$).

from which and equation 2 we get the same expressions as before for ξ and z, and the same numerical results for P and B'. The only different quantity is T, whose equation becomes

$$T = \frac{Ak(\beta - \alpha)^2 + B'(\beta + k\alpha)(k\beta + \alpha)}{\alpha\beta(\beta + k\alpha)(1 + k)} \tag{16}$$

In either case, when $B = B' = 0$, as in the experiments with creatinine,

$$P = \frac{A(\beta + k\alpha)}{\alpha\beta(1 + k)} \tag{17}$$

and

$$T = \frac{Ak(\beta - \alpha)^2}{\alpha\beta(1 + k)(\beta + k\alpha)}. \tag{18}$$

The tissue volume of creatinine given by equation 18 is smaller than the volume calculated by equation 22 of the creatinine paper (see table 4). It

TABLE 1

Protocol of experiment 3, dog 4, September 19, 1935

EXPERIMENTAL DETAILS	URINE			PLASMA	
	Interval of collection	Volume	Reducing substances	Time of collection	Non-fermentable reducing substances
		cc.	*mgm. per 100 cc.*		*mgm. per 100 cc.*
Dog not fed from 5:00 p.m., September 18, 1935, to 5:30 p.m., September 19, 1935. Intravenous injection of 67.1 cc. of 50.35 per cent solution of *d*-xylose (Pfanstiehl) in distilled water, from 9:28 to 9:28½ a.m. Water was given by stomach tube at 8:25 a.m. 250 cc., 10:05 a.m. 100 cc., 11:03 a.m. 200 cc., 12:30 p.m. 200 cc., and at 3:30 p.m. 200 cc. At 9:28 a.m., September 20, 1935, 400 cc. of urine containing 288 mgm. of reducing substance per 100 cc., were obtained by catheter. No urine was voided during the night	8:27– 9:27	49.0	79	8:56	10.9
	9:27– 9:43	181.5	2165	9:43¾	561.0
	9:43– 9:58	132.0	2281	10:01	448.0
	9:58–10:13	84.0	2964	10:13½	355.0
	10:13–10:28	42.0	4680	10:28¼	287.0
	10:28–10:58	58.5	4558	11:00½	193.0
	10:58–11:28	37.8	5021	11:28½	160.0
	11:28–11:58	36.6	3505	11:58½	118.0
	11:58–12:28	37.0	2403	12:29	82.0
	12:28– 1:28	78.2	1468	1:28½	58.0
	1:28– 2:28	66.0	840	2:28½	32.0
	2:28– 3:28	19.0	1326	3:28½	23.0
	3:28– 4:28	46.0	369	4:29	19.5
	4:28– 5:28	88.2	110	5:29	16.5

should be borne in mind that the old solution led to an equilibrium between plasma and tissues of the form $lim\ (z/\xi) = 1$, whereas the new solution arrives at one of the form $lim\ (z/\xi) = (c/a) > 1$. Therefore, if the quantitative relations are to be satisfied, the new formulation, postulating a final greater concentration in the tissue volume, must give a smaller tissue volume. The equation first given for the volume of distribution (Dominguez, 1934a) yields the largest volume possible.

The experiments to be reported in the next paragraph supply the necessary data to calculate a, b, α, β and A, and these together with the known quantity injected, G, determine uniquely the derived quantities c, γ, B and the volume P.

Experiments. Eight experiments were performed in four mongrel female dogs, the protocol of one of which is shown in table 1. The aqueous solution of *d*-xylose (Pfanstiehl) was sterilized in the autoclave the day before the injection, and its concentration determined in the fluid remaining after the injection. The average concentration was 40.75 grams of xylose in 100 cc. of solution, and the mean injection time into the jugular vein 2.33 minutes. The intravenous injection was very well tolerated by the dogs. In one instance the animal shortly after the injection vomited a watery mucus containing practically no reducing substances. In another instance a marked flush appeared in the mucous membranes of the mouth and tongue and in the skin of the thorax and abdomen, which disappeared in about two minutes. No other ill effect was noted in the experiments.

The chemical methods are essentially those already published (Dominguez and Pomerene, 1934). The mean rate of excretion of endogenous reducing substances, and the mean concentration in the plasma of endogenous non-fermentable reducing substances are shown in table 2, together with their standard errors. The average rate of excretion of total reducing substances in a given interval minus the mean rate of excretion of endogenous reducing substances, represents the "observed" rate of excretion of xylose, η, and similarly for the plasma concentration ξ. The concentrations of reducing substances in plasma and urine were determined after the samples had been renumbered by an independent worker in order to avoid bias. The weight of the dogs, quantity of xylose injected, G, and other data are shown in table 3.

Computations. The constants were calculated by the method used in the creatinine paper, with the result shown in table 3. The excretion constant, A, the per cent excretion and the exponents α and β are evidently independent of the quantity of xylose injected. The fitting of the curves has not been in general as satisfactory as that of the creatinine experiments, because of a few systematic irregularities in the raw data. The excretion in the first fifteen minutes is in more than half of the experiments somewhat deficient, and becomes overcompensated in the next hour or so, without any corresponding change in the plasma curve. It is conceivable that, upon the rapid injection of a concentrated solution of xylose, a visceral vaso-constriction of short duration sets in, succeeded by a vaso-dilatation of longer duration. Indeed a phenomenon of this sort has been described in urea experiments (Kay and Sheehan, 1933), but we have no direct evidence of it. The plasma curve on the other hand lowers so much faster than that of creatinine that many points fell below 30 mgm. per 100 cc., in a region too close to the endogenous level to be dependable. In order to overcome these difficulties and obtain more reliable mean values seven experiments were grouped (no. 2 excepted) taking the means of experiments 4, 5 and 6 as standard of reference. All the ξ points were first

TABLE 2

Endogenous non-fermentable reducing substances (mean values)

DOG NUMBER	CONCENTRATION IN PLASMA	NUMBER OF OBSERVATIONS	STANDARD ERROR OF MEAN	RATE OF EXCRETION	NUMBER OF OBSERVATIONS	STANDARD ERROR OF MEAN
	mgm. per 100 cc.			*mgm. per min.*		
1	9.60	3	0.4359	0.3091	3	0.0419
2*	10.28	6	0.3380	0.5587	6	0.0781
3	10.58	5	0.8748	0.3299	7	0.0152
4	9.79	9	0.4198	0.5019	7	0.0209

The values for the rate of excretion correspond really to reducing substances only, because, the animals having no glycosuria, no fermentation was carried out in the urine.

* This dog was identified as dog 3 in the creatinine paper (Dominguez, Goldblatt and Pomerene, 1935).

TABLE 3

Plasma concentration, ξ, and rate of output, η, of xylose

$$\xi = ae^{-\alpha t} + be^{-\beta t}, \eta = A\,\xi$$

Experiment number	Date	Dog number	Weight	G xylose injected	A	a	α	b	β	$\int_0^\infty \eta \cdot dt$	Xylose excreted in 24 hours
			kgm.	*grams*	*100 cc. per min.*	*mgm. per 100 cc.*	$(hours)^{-1}$	*mgm. per 100 cc.*	$(hours)^{-1}$	*per cent of G*	*per cent of G*
1	6-6-35	2	16.4	16.4	0.372	210.9	0.684	150.4	1.612	54.6	51.2
2	7-17-35	3	17.6	17.6	0.260	310.1	0.533	163.5	1.750	59.9	59.0
3	9-19-35	4	16.6	33.8	0.408	491.2	0.694	206.3	1.674	60.3	61.4
4	6-28-35	3	18.3	54.6	0.364	903.7	0.624	237.8	1.791	63.2	64.4
5	1-30-36	4	20.7	58.0	0.371	1062.3	0.633	292.6	1.507	71.8	66.8
6	2-12-36	4	20.5	59.3	0.377	1019.8	0.628	324.2	2.123	68.5	60.8
7	10-1-35	1	16.2	69.0	0.362	1192.2	0.639	575.6	1.450	71.3	71.5
8	10-9-35	4	17.2	70.0	0.357	1004.7	0.618	348.2	1.263	58.1	58.1
Mean curve....			17.98*	57.3†	0.3633‡	1003.58	0.6456§	368.38	1.668	67.47	

* Mean weight of the dogs in all experiments except no. 2.

† Mean G from experiments 4, 5, and 6 (see text).

‡ Mean A from all raw data except those of experiment 2 (see fig. 1).

§ Mean of the numbers in the column, except 0.533 of experiment 2.

The remaining figures of the "mean curve" were obtained by fitting equation 4 to the grouped data (see text) and not by averaging the numbers in the corresponding columns. Neither the mean of the last column (61.6) nor the proportion excreted corresponding to the mean curve (67.47) are significantly different from the mean of the integrals $\int \eta \cdot dt$, 63.45 per cent, standard error 2.85.

multiplied by the common A (=0.363, see fig. 1) and then the $A\xi$ and η points of each experiment were multiplied by a suitable factor. After this reduction it was easy to determine the mean position of the points, and the curve fitting them. The result is illustrated in figure 3. See also table 3, "mean curve."

It is obvious that with this method of calculating a mean curve the absolute residuals are increased or decreased in magnitude according as the reducing factor is greater than or less than one, but the relative residuals are not changed. The calculation of these residuals from the means shows that the average relative deviation of the η points is 3.35 per cent, irrespective of sign, while that of the ξ points is 7.87 per cent. The larger ξ

TABLE 4

Volume of distribution and its two components, computed as water in per cent of the body weight

SUBSTANCE	ANIMAL	UTILIZATION CONSTANT	V	P	T
Xylose	Dog	0.175*	25.6	23.3	2.4
		0.175†	29.9	23.3	6.6
Creatinine‡	Dog	0	63.1	28.3	34.8
Sodium chloride§	Cat	0	59.0		
Sodium thiocyanate¶	Man, horse, dog, rabbit	0	29.7		
Chloride‖	Rabbit, dog, monkey	0	27.0		

V = volume of distribution, P = plasma equivalent, T = tissue volume.

* Milligrams of xylose per minute per unit plasma concentration.

† Milligrams of xylose per minute per unit tissue concentration.

‡ Dominguez, Goldblatt and Pomerene, 1935, with T calculated by equation 18 of the present paper. T by equation 22 of the creatinine paper is equal to 47.6 per cent of the body weight.

§ Hetherington, 1931.

¶ Calculated from the data of Crandall and Anderson, 1934.

‖ Harrison, Darrow and Yannet, 1936.

residuals occur at the latter part of the experiments, when the plasma concentration lowers below 30 mgm. per 100 cc.

Substituting in equation 13 the constants of the mean curve (table 3), correcting for differences in units, and using for G the value 57.326 grams, mean of the quantity injected in experiments 4, 5 and 6, which were chosen as reference for the calculation of the mean curve, we obtain $B = 0.175$. The utilization constant in these experiments is about half as large as the simultaneous excretion constant.

The mean plasma volume P is, by equation 10 or 14, 4.18 liters. The tissue volume T is 0.44 liters by equation 11, and 1.20 liters by equation 18. The volume of distribution $(P + T)$ is then either 4.62 or 5.38 liters. The

volumes computed as water are given in table 4 together with a few others from the literature. If instead of 17.98 kgm., the mean weight of the dogs in experiments 4, 5 and 6 had been used, namely, 19.8 kgm., the per cent value of the volumes would have been somewhat smaller.

Discussion. Although the experiments give a unique numerical value to the utilization constant B, an ambiguity remains because it cannot be decided on the strength of these experiments alone whether a utilization rate of 0.175 mgm. of xylose per minute per unit concentration refers to the concentration in the plasma or to that in the tissues. The ambiguity becomes apparent in the double value of the tissue volume and consequently of the volume of distribution. Having no grounds for choosing one or the other value we shall regard them as first approximations.

The data of table 4 show that the volume of distribution of xylose is comparable to that of thiocyanate and also to the equivalent plasma volume of creatinine. It is also comparable to the volume of distribution of chloride by the method of Harrison, Darrow and Yannet (1936), but much smaller than that estimated by the method of Hetherington (1931).

We know of no other method of estimating the volume of distribution of a substance which is rapidly excreted and partly utilized. For a rapidly excreted inert substance the calculation of Crandall and Anderson (1934) is not applicable while that of Hetherington may give in actual computation quite erratic results. For these reasons we have not included in table 4 the volumes of distribution of sulphate and sucrose estimated by Lavietes, Bourdillon and Klinghoffer (1936) and Bourdillon and Lavietes (1936).

For extended discussions of the nature of the volume of distribution of chloride the reader is referred to the book of Peters (1935) and to the recent review by Fenn (1936).

With regard to the relation between the constants of utilization and excretion of xylose and the characteristic ratio of McCance and Madders (1930) we should like to point out that it holds in the form given by one of us (Dominguez, 1934b), even though the assumption is untenable that the plasma is in equilibrium with the tissues from the beginning of the injection.

SUMMARY AND CONCLUSIONS

1. The elimination of *d*-xylose after a single rapid intravenous injection has been investigated in a group of dogs of comparable size.

2. The time change of the plasma concentration of xylose after the injection can be represented by the equation $\xi = ae^{-\alpha t} + be^{-\beta t}$, if the disturbances in the first few minutes are neglected.

3. The rate of excretion of xylose in the course of these experiments has been found to vary linearly with the instantaneous concentration in the

plasma, the proportionality factor (excretion constant of xylose) having in 7 out of 8 experiments the mean value 0.363 mgm. per minute per unit plasma concentration.

4. The mean proportion of xylose excreted is, from the curves fitted to the data, 63.45 per cent of the quantity injected, with a standard error of 2.85.

5. The constants α and β, the ratio b/a, the rate of excretion per unit plasma concentration, and the proportion of xylose excreted, are independent of the quantity injected.

6. The constant of integration a is proportional to the quantity injected, the only arbitrary quantity in these experiments.

7. The rate of utilization per unit plasma (or tissue) concentration is a constant independent of the quantity injected. Before equilibrium it is not known whether the rate of utilization is proportional to the plasma concentration or proportional to the tissue concentration. The numerical value of the constant is the same in both cases, its mean value in 7 out of 8 experiments being 0.175 mgm. per minute per unit plasma (or tissue) concentration.

8. The volume of distribution of xylose estimated as water is equal to 26 or 30 per cent of the body weight, depending on the assumed rate of utilization. The equivalent plasma volume represents 23 per cent of the body weight, and is independent of the rate of utilization and excretion.

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SEASONAL VARIATIONS IN THE NUMBER OF PLATELETS IN ARTERIAL, VENOUS AND CUTANEOUS BLOOD OF MAN[1]

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Received for publication October 13, 1936

In normal men there are significant differences between blood platelet counts done almost simultaneously on arterial, venous and cutaneous blood; these differences are markedly accentuated under pathological conditions (1). The evidence here presented further indicates that from the winter to the spring of the year significant changes occur in the total number of platelets in the blood, and in the relative platelet content of blood from various vessels.

METHODS. Counts of the platelets and erythrocytes by methods described in detail elsewhere (2) were done *directly* on blood obtained: *a*, by puncture of one of the antecubital veins, and *b*, of the radial artery using 27 gauge needles and a finely graduated syringe containing the diluting fluid; *c*, by incision of the skin of the finger. All determinations were performed in the order stated, on the same extremity, one immediately after the other, between the hours of two and four in the afternoon, after a period of 20 to 30 minutes' body rest, in a room at a temperature between 18° and 21°C. The subjects were 28 white, male medical students (ages: 23–28). All counts were taken in Philadelphia (altitude: −10 to +440 feet in relation to sea level) between February 10 and May 12, 1936. The mean weekly temperatures of the atmosphere for this period, as calculated from mean daily temperatures obtained from records of the Philadelphia station, Weather Bureau, U. S. Department of Agriculture, were, beginning February 5: −4°, −1°, −2 2°, +4.5°, +5.5°, +11.°, +10.°, +13.8°, +7.5°, +8.2°, +11.5°, +11.°, +20.3°, +20.8°C.

The methods employed for calculating the descriptive constants and for testing the significance of differences are detailed in Dunn's (3) review. No single arbitrary standard of probability was fixed as a measure of significance for all the differences found. In comparing counts done *at the same time* on blood from continuous, neighboring vessels, under conditions of rest, it would be extremely difficult to establish a significant difference be-

[1] Aided by a grant from the J. Ewing Mears Research Fund.

tween those counts if one were to adhere rigidly to the standard generally accepted to test the significance of differences: the chances that the difference is due to random sampling must not be greater than 1 in 100. When comparing counts done at the same time on arterial and venous blood, one is not comparing strictly independent, static entities. Venous blood is being continuously influenced by arterial blood. An arteriole-venule anastomosis may shunt much of the blood from an artery to a vein away from the capillaries where a change in its composition might have taken place, had the blood passed through them. These reasons seemed to justify considering a difference between arterial and venous blood counts done *at the same time* as probably significant, when the chances of its arising by sampling were not greater than 8 in 100 $\left(\frac{\bar{x}}{\text{P. E.}} = 2.6\right)$.[2] When comparing counts done *at different times* on blood from the same vessels, it was obviously necessary to adhere to a stricter standard of judging probability because of the greater multiplicity of factors that may have intervened, between the two determinations, to alter the frequency distribution of the counts. Under these circumstances, differences were considered significant only when the probability of their arising by sampling was not greater than 1 in 100 $\left(\frac{\bar{x}}{\text{P. E.}} = 3.7\right)$.

RESULTS. In table 1 are summarized the findings and in table 2 the differences between the findings in the two groups. Five of the individuals in the winter group were re-examined at the time counts were being done on the second group; the first and second series of counts on these 5 subjects are listed in table 3.

Platelets. 1. In the winter, counts on arterial blood were significantly higher (+19 per cent) than on venous blood. 2. In the winter, counts on arterial and venous blood were markedly and significantly higher (+85 per cent and +55 per cent respectively) than on cutaneous blood; in the spring, the differences between these counts were not significant. 3. Counts done on arterial and venous blood in the winter were significantly higher (+69 per cent and +50 per cent respectively) than in the spring. Counts on cutaneous blood did not significantly differ whether done in the winter or in the spring.

Erythrocytes. 1. There were no significant differences between the arterial and venous blood counts either in winter or in the spring. 2. In the winter, counts on arterial and venous blood were slightly, but not significantly, higher (+6.4 per cent and +8.4 per cent respectively) than on cutaneous blood. In the spring the differences between these counts were

[2] The probability equivalents of $\frac{\bar{x}}{\text{P.E.}}$ values are tabled on p. 341 of Dunn's review (3).

small and insignificant. 3. Counts on arterial and venous blood in the winter were higher (+6.6 per cent and +9.1 per cent respectively), but not significantly so, than in the spring. There were no significant differences between the counts on cutaneous blood done in the winter and in the spring.

Erythrocyte platelet (E/P) ratio. Since the number of erythrocytes underwent no significant changes, the significant differences in this ratio were simply a reflection of variations in the platelet count in the two seasons.

TABLE 1

Results of the blood counts in 28 white adult males, fourteen examined during the winter and 14 during the spring

	ARTERIAL			VENOUS			CUTANEOUS		
	Plate. $\times 10^3$	Eryth. $\times 10^6$	E/P	Plate. $\times 10^3$	Eryth. $\times 10^6$	E/P	Plate. $\times 10^3$	Eryth. $\times 10^6$	E/P
Winter (February 10 to March 16)									
Mean	495,0	5,410	11.6	417,0	5,510	14.2	268,0	5,080	19.9
Probable error	±22,1	±106	±0.58	±21,1	±142	±0.88	±10,2	±129	±1.08
Standard deviation	118,0	570	3.13	113,0	760	4.72	55,0	694	5.8
Minimum	270,0	4,400	6.8	230,0	4,550	7.8	180,0	4,145	12.6
Maximum	670,0	6,460	17.7	690,0	7,100	23.0	358,0	6,820	33.4
Spring (May 4 to 12)									
Mean	293,0	5,070	17.5	278,2	5,050	19.4	264,1	5,131	19.8
Probable error	±11,2	±115	±0.8	±12,5	±103	±1.2	±11,0	±90	±1.0
Standard deviation	60,3	617	4.4	67,1	552	6.8	59,0	486	5.5
Minimum	210,0	4,225	11.0	160,0	4,025	11	174,0	4,260	12.0
Maximum	410,0	6,075	24.0	370,0	5,800	39	348,0	6,090	31.0

Figures for platelets and erythrocytes as per cubic millimeters of blood.
E/P = erythrocyte-platelet ratio.
Mean of daily temperatures for the winter period: 3°C.
Mean of daily temperatures for the spring period: 18.5°C.

The extent of the differences just analyzed may be estimated further by a survey of table 3, in which are compared individual counts done at two different times of the year on the same subject. Platelet and erythrocyte counts done on 27 other individuals of the same age group throughout the months of April, May and June conformed generally to the level of the group examined during the spring. The seasonal differences first began to be noticed on April 2.

COMMENT. Perhaps the differences in the platelet counts between arterial, venous and cutaneous blood in the winter are in part attributable to the general effect of the external temperature. The difference between the oxygen content of arterial and venous blood drawn from the arm is

greatly influenced by the temperature of the part (4). Cold slows down the circulation and magnifies the difference while heat has the reverse effect (4). What is true of differences in oxygen content of the blood, however, may not necessarily apply to formed elements. The higher platelet

TABLE 2

Differences between the counts done on blood collected: 1 from different vessels at the same time; 2, from the same vessels at different times

	WINTER			SPRING			ARTERIAL	VENOUS	CUTANEOUS
	A-V	A-C	V-C	A-V	A-C	V-C	Winter-Spring	Winter-Spring	Winter-Spring
Platelets									
Difference ($\bar{x}$) and P. E. of difference $\times 10^2$	*78,0* ±30,6	*227,0* ±24,4	*149,0* ±22,6	14,8 ±16,2	28,9 ±15,7	14,1 ±16,2	*201,9* ±24,8	*138,7* ±24,5	3,8 ±15,1
$\frac{\bar{x}}{\text{P. E.}}$	2.54	9.3	6.5	0.91	1.84	0.87	8.1	5.6	0.25
Probability of diff. arising by sampling..	8%	0	0	53%	21%	54%	0	0	86%
Erythrocytes									
Difference ($\bar{x}$) and P. E. of difference $\times 10^3$	100 ±178	330 ±166	430 ±191	20 ±153	61 ±147	81 ±136	340 ±157	460 ±176	51 ±157
$\frac{\bar{x}}{\text{P. E.}}$	0.56	1.9	2.2	0.13	0.41	0.59	2.16	2.61	0.32
Probability of diff. arising by sampling	71%	19%	13%	92%	77%	68%	14%	7%	82%
E/P Ratio									
Difference ($\bar{x}$) and P. E. of difference	*2.6* ±1.0	*8.3* ±1.1	*5.7* ±1.2	1.9 ±1.4	2.3 ±1.2	0.4 ±1.5	*4.1* ±0.9	*5.2* ±1.4	0.1 ±1.4
$\frac{\bar{x}}{\text{P. E.}}$	2.6	7.5	4.7	1.3	1.9	0.26	4.5	3.7	0.07
Probability of diff. arising by sampling	7%	0	0	49%	19%	86%	0	1%	96%

A = arterial, V = venous, C = cutaneous, P. E. = probable error.

counts found in the winter appear to be the result of living under winter conditions for a prolonged period of time and not to transient exposure to cold weather during the day of the examination. Perhaps analogous seasonal changes occur in the gaseous content of blood from different vessels.

The slight but not statistically significant decrease in the erythrocyte count on arterial and venous blood that accompanies, in the spring, the decrease in number of platelets, suggests that blood dilution may play an important part in bringing the differences about. Changes of an inverse order, however, take place in other cellular and chemical elements of the blood, from the winter to the spring. There are increases in the percentage of reticulocytes (5) (6) and eosinophiles (7); the blood phosphorus shows a rise correlated directly with local changes in the mean daily temperature and percentage hours of sunshine, and inversely with the incidence of rickets (8).

TABLE 3

Counts on the same individual at 2 occasions

SUBJECT	DATE	ARTERIAL			VENOUS			CUTANEOUS			MEAN TEMP. OF DAY
		Plate. $\times 10^3$	Eryth. $\times 10^6$	E/P	Plate. $\times 10^3$	Eryth. $\times 10^6$	E/P	Plate. $\times 10^3$	Eryth. $\times 10^6$	E/P	
C. S.	Mar. 13	470,0	5,050	10.7	420,0	5,250	12.5	235,0	4,615	19.6	3.3
C. S.	May 7	210,0	4,225	20.1	235,0	4,400	18.7	180,0	5,300	27.7	18.8
J. W.	Feb. 14	436,0	6,460	14.8	440,0	6,500	14.7	271,0	4,840	18.3	0.5
J. W.	May 4	275,0	4,840	17.6	160,0	4,550	28.4	210,0	5,010	23.8	16.6
J. O'K.	Mar. 13	420,0	5,650	13.4	480,0	5,150	10.7	334,0	5,765	17.2	3.3
J. O'K.	May 8	315,0	5,875	18.6	330,0	5,225	15.8	260,0	5,760	22.1	25.0
A. Z.	Feb. 13	320,0	5,400	16.8	240,0	5,600	23.0	180,0	5,300	29.0	3.8
A. Z.	May 8	255,0	5,000	19.6	165,0	5,575	33.7	174,0	5,450	31.3	25.0
W. O.	Feb. 17	550,0	4,400	8.0	490,0	4,600	9.4	317,0	4,430	13.9	4.4
W. O.	May 6	250,0	4,200	16.8	220,0	4,500	20.4	310,0	4,820	15.5	17.2

Figures for platelets and erythrocytes as per cubic millimeters of blood.
E/P = erythrocyte-platelet ratio.
Temp. = degrees centigrade.

During the spring there is a greater frequency in the attacks of purpura and thrombopenia than at other times of the year (9). Perhaps when the decrease in the number of platelets in the spring occurs in the blood of individuals who usually have a lower than normal platelet count, a marked thrombopenia results. Subject A. Z. (venous blood, table 3) exemplifies this change in a moderate degree. There are individuals who develop thrombopenic purpura upon ingestion of certain substances, the sequence of events sometimes occurring only in the spring or summer of the year (10). A vernal thrombopenia may supply the ground necessary for the development of the type of purpura complicating food allergy.

Since any direct method of obtaining cutaneous blood for platelet count-

ing involves destruction of a certain proportion of the platelets (2), the lower number of platelets on cutaneous as compared with venous blood, in the winter, may seem to be due mainly to differences in the method of obtaining samples. If this were so, however, one would expect to find approximately the same ratio between the platelet content of cutaneous and arterial or venous blood in the spring, as was found in the winter. Such was not the case; in the spring, platelet counts on cutaneous blood were not significantly different from those on venous blood, and in many individual instances were even higher (table 3).

Seasonal variations may account in part for much of the discrepancy between averages for the number of platelets in normal men, found by observers in various portions of the world, working under widely different climatic conditions. Changes in altitude alone influence the number of platelets in venous and cutaneous blood more markedly than the number of erythrocytes (11) (12).

SUMMARY

In the winter, the number of platelets in arterial and venous blood from the upper extremity in man is significantly higher than in the spring; there are no significant differences between counts on cutaneous blood in the two seasons. In the winter, the number of platelets in arterial blood is significantly higher than in venous blood; it is also significantly higher in venous than in cutaneous blood. In the spring, there are no statistically significant differences between the means of platelet counts on blood from the three sources. There are no statistically significant differences between the erythrocyte counts in the 2 seasons.

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METABOLIC CHANGES IN LIVER, KIDNEY AND ANTERIOR HYPOPHYSIS OF PREGNANT, PARTURIENT AND LACTATING RATS

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Received for publication December 10, 1936

The metabolism of the liver (15) and anterior hypophysis (13) of the rat changes during the oestrus cycle and after spaying. Under the same conditions the renal cortex shows no alteration in its respiratory rate. Cytologic (4, 10) weight (1) and gonad-stimulating potency changes (8, 11) in the anterior hypophysis also occur in these same phases of reproduction. Since cytologic (11) and gonad-stimulating potency changes in the anterior hypophysis are found in (7) and during pregnancy it seemed important to study the metabolism of this gland and other tissues under such conditions.

This report is concerned with the rates of oxygen consumption and R. Q. of rat liver and renal cortex on the 18th day of pregnancy, at parturition and on the 21st day of lactation. The respiratory and aerobic and anaerobic glycolytic rates of the anterior hypophysis of the same animals were studied.

METHOD. The breeding, diet and care of the stock of rats have been described by Andersen and Kennedy (1932). When 80 to 90 days old, normal females were mated. The pregnant rats were divided into 3 series and studied at the 18th day of pregnancy, at parturition, and on the 21st day of lactation. Positive matings were determined by the presence of a vaginal plug. Parturition was recognizable clinically from the nesting behavior of the animals and the uterine contractions easily visible through the abdominal wall. In 2 instances one fetus was born before the mother was sacrificed. The stage of labor was confirmed at autopsy. In several cases a fetus had partly passed through the dilated cervix. To equalize the lactation stimulus each mother nursed 7 young during the 21 day period. Infected animals, noted at autopsy, were excluded from the experiments.

The age of the rats ranged from 101 to 141 days at the time of the metabolic studies. They were sacrificed by a blow on the head. The preparation of the tissues and the methods for studying their metabolism have been described elsewhere (9, 13, 15). The oxygen consumption and CO_2 production of the same tissue slices were studied with a differential volu-

meter (16). The respiratory and aerobic and anaerobic glycolytic rates of a single anterior pituitary were measured with a respirometer that has been described (14).

RESULTS. *Liver and kidney.* The individual data of the respiratory rates and quotients of liver and renal cortex in pregnant, parturient and lactating rats are recorded in figure 1. A summary of these results is found in table 1, which includes the means, their probable errors and the standard deviations. Table 2 shows the differences of the means, their probable errors and the ratio of the difference to its probable error. When the ratio is over 3 (statistically significant) the difference is italicized.

Respiratory rate. The highest liver respiratory rates in any of these states were those of the 18 day pregnant rats. On the other hand, the

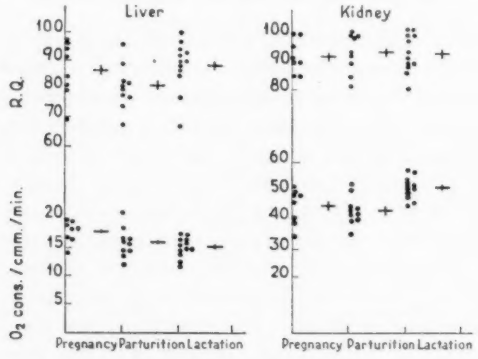

Fig. 1. Scatter chart of individual respiratory rates and quotients of liver and kidney from 18-day pregnant, parturient and 21-day lactating rats. The R.Q. of the livers of pregnant and lactating rats were higher than those of parturient ones. There were no R.Q. changes in the kidney. The respiratory rates were highest in the livers of pregnant and in kidneys of lactating rats.

metabolic rate of the kidney cortex was highest in the lactating rats. No significant differences were found in the respiratory rates of the livers of parturient and lactating rats, nor in the kidneys of pregnant and parturient rats.

Previous studies showed that the mean rates of oxygen consumption of oestrual, dioestrual and spayed rat liver were 17.7 ± 0.24, 14.9 ± 0.25 and 13.3 ± 0.36 cmm./gm./min. respectively. In the present series only the livers of pregnant rats had a mean rate as high as the oestrual one, namely, 17.8 ± 0.5. The livers of the parturient and lactating rats fall in the dioestrus range, being 15.9 ± 0.57 and 15.2 ± 0.39 respectively as compared with 14.9 ± 0.25 for the latter. On the other hand the mean rates of liver metabolism in pregnant, parturient and lactating, like those of oestrual and dioestrual rats, were higher than those of spayed ones.

The mean rates of respiration of rat renal cortex in oestrus, dioestrus and after spaying were 49.1±0.8, 49.7±1.4 and 47.4±0.8 respectively (5). In the present series the kidneys of the lactating rats had a significantly higher mean respiratory rate than those of the spayed rats, the mean difference being 4.3±1.1 cmm. O_2/gm./min., with a significant ratio of difference to probable error of 4.0. On the other hand the mean metabolic rates of the kidneys of oestrual and dioestrual rats were significantly higher than those of pregnant or parturient rats. In other words, the metabolic rates of

TABLE 1

REPRODUCTIVE PHASE	NO.	LIVER				KIDNEY			
		O_2 Consumption cmm./gm./min.		R.Q.		O_2 Consumption cmm./gm./min.		R.Q.	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Pregnancy (18 days)..	8	17.8±0.5	2.1	0.864±0.021	0.09	44.9±1.4	5.8	0.922±0.015	0.064
Parturition..	10	15.9±0.57	2.7	0.812±0.016	0.076	43.6±1.4	6.6	0.938±0.014	0.062
Lactation (21 days)..	12	15.2±0.39	2.0	0.884±0.017	0.086	51.7±0.7	3.6	0.937±0.014	0.071

TABLE 2

Metabolic differences of liver and kidney in pregnancy, parturition and lactation

COMPARISON OF	LIVER				KIDNEY			
	O_2 Consumption cmm./gm./min.		R.Q.		O_2 Consumption cmm./gm./min.		R.Q.	
	Diff.	Diff. / P.E. Diff.	Diff.	Diff. / P.E. Diff.	Diff.	Diff. / P.E. Diff.	Diff.	Diff. / P.E. Diff.
Pregnancy-parturition....	1.9±.76	2.5	0.052±0.026	2.0	1.3±2.0	0.7	0.016±0.02	0.8
Pregnancy-lactation.....	2.6±.63	4.1	0.020±0.027	0.7	6.8±1.6	4.2	0.015±0.02	0.8
Parturition-lactation.....	0.7±.69	1.0	0.078±0.023	3.2	8.1±1.6	5.1	0.001±0.02	0

renal cortex of oestrual, dioestrual, spayed and lactating rats were higher than those of pregnant or parturient ones. Since the kidney metabolism is not altered during the oestrus cycle, the normal cyclic change of the female rat, the results indicate that the metabolic rates of the kidneys of the pregnant and parturient rats were depressed rather than that of those of the former were in a stimulated state.

Respiratory quotient. The R.Q. of the liver was highest in the lactating and lowest in the parturient rats. The only statistically significant difference was found in comparison of these states. However comparison of

these R.Q. with those of livers of oestrual, dioestrual and spayed rats, which were 0.79±0.014, 0.82±0.017 and 0.77±0.012 respectively, show that the R.Q. of the liver of lactating rats was higher than those of oestrual, dioestrual, spayed or parturient rats. In pregnancy the liver R.Q. likewise seems to be elevated but the statistical significance of this increase was not as decisive as that found in similar comparisons with the livers of lactating rats. These results show that the metabolism of the liver of lactating or pregnant rats is qualitatively different from that of

TABLE 3

Summary of respiratory and glycolytic rates of anterior pituitary of rat

Pregnancy, parturition and lactation

STAGE	NO.	O_2 CONSUMPTION Cmm./gm./min.		AEROBIC GLYCOLYSIS CO_2 Cmm./gm./min. Equivalent to acid production		ANAEROBIC GLYCOLYSIS CO_2 Cmm./gm./min. Equivalent to acid production	
		Mean	St. dev.	Mean	St. dev.	Mean	St. dev.
Pregnancy (18 days)	8	12.8±0.54	2.3	6.2±0.57	2.4	19.4±1.27	5.4
Parturition	9	20.8±1.18	5.3	5.5±0.47	2.1	16.6±1.20	5.4
Lactation (21 days)	10	15.3±0.32	1.5	4.9±0.55	2.7	16.0±0.79	3.9

TABLE 4

Metabolic differences of anterior pituitary of rat in pregnancy, parturition and lactation

COMPARISON OF	O_2 CONSUMPTION Cmm./gm./min.		AEROBIC GLYCOLYSIS CO_2 Cmm./gm./min. Equivalent to acid production		ANAEROBIC GLYCOLYSIS CO_2 Cmm./gm./min. Equivalent to acid production	
	Diff.	Diff. / P.E. Diff.	Diff.	Diff. / P.E. Diff.	Diff.	Diff. / P.E. Diff.
Pregnancy-parturition	*8.0*±1.3	6.2	0.7±0.74	0.9	2.8±1.75	1.6
Pregnancy-lactation	*2.5*±0.63	4.0	1.3±0.79	1.6	3.4±1.50	2.3
Parturition-lactation	*5.5*±1.22	4.5	0.6±0.73	0.8	0.6±1.44	0.4

oestrual, dioestrual, spayed or parturient ones. An increase in liver R.Q. has been found in infected rats (15). However, no evidence of infection was found in these animals at autopsy in which the gross organs and middle ears, the most common site of infection, were examined in every case. All the respiratory measurements were made in 0.2 per cent glucose-Ringer solution so that it cannot be stated that the high R.Q. in the livers of the lactating and pregnant rats was due to an increased oxidation of glucose. Since the diet was the same for all the animals the liver R.Q. changes suggest either greater oxidation of glucose or incomplete oxidation of proteins.

Although the kidneys of the pregnant, parturient and lactating rats showed quantitative differences in respiration, indicated by the changes in metabolic rate, there were no qualitative differences. The R.Q. of the renal cortex from animals in the reproductive states studied in this report was not significantly different from that of oestrual, dioestrual or spayed animals. The mean R.Q. of the last group were found to be 0.96 ± 0.012, 0.96 ± 0.012 and 0.92 ± 0.016 respectively as compared with 0.92 ± 0.015, 0.94 ± 0.014 and 0.94 ± 0.014 in this series.

Anterior hypophysis. The mean respiratory and aerobic and anaerobic glycolytic rates of the anterior pituitaries of 18-day pregnant, parturient, and 21-day lactating rats are presented in table 3, as well as their probable errors and standard deviations. Table 4 shows the differences between the means and the probable error of the difference as well as the ratio of the

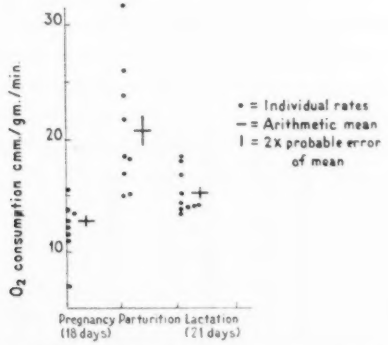

Fig. 2. Scatter chart of individual rates of oxygen consumption of anterior pituitaries of 18-day pregnant, parturient and 21-day lactating rats. The highest metabolic rates occurred at parturition. Those of lactating rats were intermediate.

difference to the probable error. When this ratio is over 3 (statistically significant) it is italicized. Figure 2 is a scatter chart of the individual rates of oxygen consumption of the glands of the pregnant, parturient and lactating rats. The means and their probable errors are also indicated.

There were differences between the mean respiratory but not the glycolytic rates of the anterior pituitaries of the pregnant, parturient and lactating rats. The highest rates were found at parturition and the lowest on the 18th day of pregnancy. The respiratory rates of the glands of the lactating rats were intermediate between those of pregnancy and parturition. Among 90 anterior pituitaries studied in various reproductive states other than parturition, namely, in prooestrus, oestrus, dioestrus, spayed, pregnant and lactating rats the highest rate of oxygen consumption was 18.6 cmm./gm./min. Five of 9 measurements of the anterior pituitary respiration at parturition showed respiratory rates above this.

A comparison of the above observations with the metabolic rates of anterior pituitaries of prooestrual, oestrual, dioestrual and spayed rats is presented in figure 3. The mean respiratory rates and their probable errors are indicated. The results are arranged in chronological sequence in the oestrus cycle and after pregnancy. In addition, the changes in the cytological characteristics of the anterior pituitary are plotted. The cytological data are taken from the reports of Wolfe (10), Wolfe and Cleveland (11), and Ellison and Wolfe (6). According to these authors the most labile cell type under these conditions was the granular basophil. The changes in their percentage in the various reproduction phases are plotted

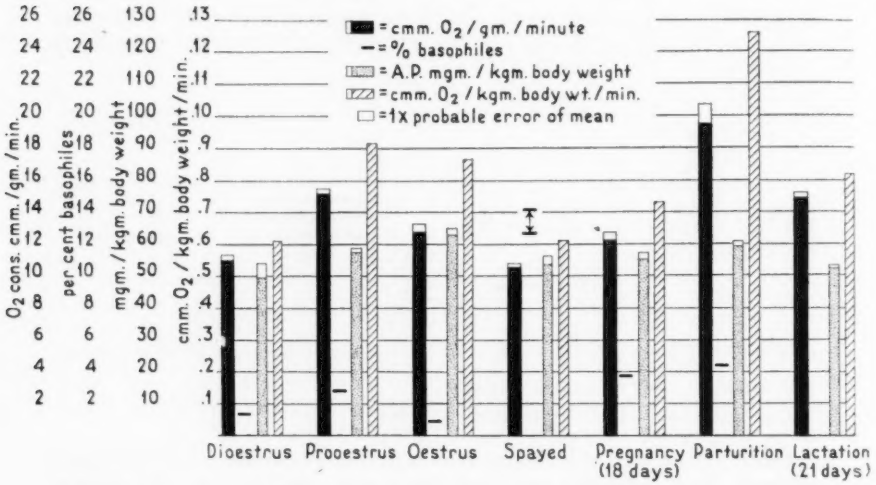

Fig. 3. Comparison of the mean respiratory rates, percentage of granular basophils (Wolfe et al.), total anterior pituitary weights per kilogram body weight. There is no correlation between granulation and metabolism. The total metabolism is greatest at parturition > prooestrus > oestrus > lactation > pregnancy > dioestrus or spayed.

with the metabolic rates of the anterior pituitary in the same states. The data may not be strictly comparable because the age of the animals used for the cytological data was not presented. Since Wolfe has shown that the differential count of the anterior pituitaries of immature animals varies from that of adults, differences may exist between adult rats of different ages. Comparisons have also been made with the weight changes of the pituitary of the same age and from the same colony (1, 3).

Relation of metabolism to cell types of the anterior pituitary. The mean respiratory rate of the anterior pituitary in the oestrus cycle was highest at prooestrus, when the percentage of granular basophils was greatest,

namely, 2.8 per cent. The mean respiratory rate was lowest in dioestrus when the mean percentage, 1.3, of granular basophils was intermediate. On the other hand in oestrus the mean respiratory rate was intermediate between prooestrus and dioestrus while the percentage of granular basophils was lowest, namely, 0.9 per cent. This result may not be inconsistent with a correlation between the percentage of granular basophils and the anterior pituitary rate of oxygen consumption. It was found that respiratory rates of the gland in oestrus rats showed considerable variability with overlapping between the prooestrus and dioestrus levels (13). Since oestrus was not as sharply defined, temporally, as prooestrus, the measurements in the oestrus series may have included some prooestrus animals. Under such conditions the metabolism of the oestrus and dioestrus pituitaries might be the same. In that case the pituitary metabolism in the oestrus cycle would be directly correlated with the percentage of granular basophils (see fig. 3).

However, comparison of the metabolism of the anterior hypophysis of spayed rats (spayed 6 weeks) with the percentage of granular basophils showed a decided breakdown of any such correlation. The mean metabolic rate of the anterior pituitary was about the same as that of dioestrus rats, but the percentage of granular basophils in the spayed rats according to Wolfe was 14.2 after 30 days and 12.8 after 60 days, while that of dioestrus rats was 1.3.

The anterior pituitary of 18-day pregnant rats had a mean respiratory rate not significantly different from that of oestrus ones. The mean percentage of granular basophils, however, was about twice as high in the former animals. Another discrepancy between pituitary metabolism and percentage of granular basophils was seen at the time of parturition. The increase in metabolism as compared with the 18-day pregnant rat's pituitary was 62 per cent, while the increase in percentage of granular basophils was only 18 per cent. However, there were only 2 rat pituitaries studied in the series of Wolfe and Cleveland (11) on the 21st day of pregnancy.

These data indicate that there is no correlation between the number of granular basophils and the metabolism of the anterior pituitary of the rat. While it is possible to explain the discrepancies between the percentage of granular basophils and the pituitary metabolism by assuming 2 variables, namely, stimuli for basophilic granulation and degranulation, further information is necessary before speculation will be profitable.

Metabolic and weight changes of the anterior pituitary. It was conceivable that in pregnant, parturient and lactating rats the changes in metabolic rate of the anterior pituitary might compensate for changes in gland weight. Under certain conditions such changes might result in no alteration in the total metabolism of the gland. In a comparable series of rats of the same age and breed, the pituitaries were removed and weighed.

Figure 3 shows their arithmetic mean weights as well as the probable errors of the means. A similar series obtained in dioestrual, prooestrual, oestrual and spayed rats (1, 3), is also presented. The anterior pituitary weights are expressed as mgm./kgm. body weight. By multiplying the weight of the anterior pituitary with its rate of oxygen consumption the total metabolic rate of the anterior pituitary was obtained. The results show that the highest anterior pituitary metabolism was found at the time of parturition. The weight changes did not in any way compensate for the metabolic changes, indeed, they seemed to enhance the metabolic differences in the various phases. However, the similarity of the rate of respiration per gram per minute in prooestrus and lactating rats was somewhat disturbed when the total organ weight was considered. Because of the greater pituitary weight in prooestrual rats the total pituitary metabolism was somewhat greater than that of the lactating rats. In descending order of total pituitary metabolism the various reproductive phases may be listed as follows: parturition, prooestrus, oestrus, lactation (21 days), pregnancy (18 days), dioestrus and spayed (6 weeks).

Discussion. The literature related to basal metabolism in pregnancy has recently been reviewed by Enright, Cole and Hitchcock (5). Their own experiments in humans confirmed the findings of most other workers and led to the conclusion that the increase in metabolism was largely contributed to by the fetus. From the above observations on isolated tissues from pregnant rats it would appear that the response of the entire animal may be quite different from that of individual tissues. For example, the liver metabolic rate was high, whereas the kidney's was low. The increase in liver metabolism, using the dioestrus level as the resting level, was about 19 per cent. On the other hand according to the same standard the decrease in kidney respiration was about 6 per cent. The algebraic sum of the changes in liver and kidney metabolism, however, would tend to neutralize each other if estimated from the relative organ weight and metabolism.

The pituitary shows even more individuality and lability than either liver or kidney. At parturition, the mean respiratory rate of the anterior pituitary had increased 62 per cent as compared with that of 18-day pregnant rats, while that of the liver decreased 11 per cent and that of the kidney was unaltered. After 21 days of lactation the anterior pituitary had only a 36 per cent greater respiratory rate as compared with that of pregnant rats while the liver rate was still about 10 per cent lower and that of the kidney was 16 per cent higher. These examples demonstrate the diverse responses of different organs of the same animal at the same time. Of the 3 tissues, anterior pituitary, liver and kidney, studied in the various reproductive states, the first is the most labile in its metabolic response.

The above experiments clearly show that there were metabolic differ-

ences between the anterior pituitaries of 18-day pregnant, parturient, and 21-day lactating rats. The causes of these changes are not as apparent. They certainly are not part of general changes in metabolic processes because they occur independently of the changes in other tissues. Furthermore, the extraordinary lability, specificity, and degree of alteration of the pituitary metabolism suggests that these changes were not due to alterations in thyroid activity. Nor do the metabolic changes seem to be secondary to cytologic ones, since there is no direct correlation between the cytology and metabolism of the cells. However, the metabolic changes in the anterior pituitary are associated with functional changes in the reproductive phases of the rat.

The substance or substances responsible for the metabolic changes in the anterior lobe of the rat pituitary, affect it very rapidly and specifically. At prooestrus and parturition the marked respiratory increase occurs in from 1 to 3 days when compared with dioestrus or pregnancy respectively.

SUMMARY AND CONCLUSIONS

1. The metabolism of the liver, kidney and anterior lobe of the rat pituitary was studied in vitro. The animals were 18 days pregnant, at parturition, or had been lactating for 21 days. The age varied from 101–140 days. The rate of oxygen consumption and R.Q. of liver and kidney and the respiratory and aerobic and anaerobic glycolytic rates of each pituitary were measured. The results were compared with those found in prooestrual, oestrual, dioestrual, and spayed rats.

2. The mean respiratory rate of the liver was highest on the 18th day of pregnancy.

3. The mean respiratory rate of the kidney cortex was highest on the 21st day of lactation.

4. The mean R.Q. of the liver was higher in pregnant and lactating rats than in parturient, oestrual, dioestrual or spayed ones. These qualitative changes in liver metabolism in pregnancy and lactation indicate a greater combustion of glucose or more incomplete oxidation of protein.

5. The R.Q. of the kidney cortex did not change in these states.

6. Comparison with other reproductive states indicates that the metabolism of the kidney is depressed in pregnancy and at parturition, while in lactation it is "normal".

7. In the same animal the metabolism of individual organs may be altered in opposite directions, i.e., in pregnancy the metabolic rates of the liver and anterior hypophysis were increased, while those of the kidney were decreased.

8. Significant differences were found between the respiratory rates of the anterior pituitaries of pregnant, parturient and lactating rats. These metabolic changes were associated with differences in gland weight. There

were no significant differences in their aerobic or anaerobic glycolytic rates.

9. Arranged in decreasing order of respiratory activity the anterior pituitaries in the various reproductive states show the following mean rates of oxygen consumption in cmm./gm./min.: (1) parturition 20.8 ± 1.18; (2) lactation 15.3 ± 0.32 and prooestrus 15.5 ± 0.38; (3) oestrus 13.3 ± 0.53 and pregnancy 12.8 ± 0.54; (4) dioestrus 11.3 ± 0.36 and spayed 10.8 ± 0.20.

10. There is no direct correlation between the cytology (6, 10, 11) of the anterior pituitary of the rat and its metabolism.

11. The anterior pituitary metabolism shows greater alterations than that of liver or kidney in the various reproductive phases. Its metabolism may change in directions that are different from those of liver or kidney. The rapidity and extent of its changes suggest that the responsible mechanism or mechanisms affect it in a specific way.

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ASCORBIC ACID DURING PREGNANCY IN THE RABBIT[1]

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Received for publication December 28, 1936

Several reports have been made of a high ascorbic acid content in the corpora lutea (Bessey and King, 1933; Huszak, 1933; Giroud, Leblond and Giroud, 1934; Bourne, 1935a; Biskind and Glick, 1936). In only one (Biskind and Glick, 1936) has any attempt been made to follow the change in ascorbic acid content with the progress of pregnancy and some of the material of Biskind and Glick on the cow is admittedly inaccurately timed. The rabbit is extremely useful in this respect since ovulation occurs at 10 hours after copulation.

We were further led to undertake this investigation because of the observation of one of us (Pincus, unpublished data) that the growth of uterine ova *in vitro* appears to be accelerated by the addition of ascorbic acid to the medium. Furthermore Hall (1936) has recently published the finding that the uterus of the mouse and rat exhibit increased acidity and reducing potential during at least certain periods coincident with blastocyst growth in the uterus. During the period of blastocyst growth within it the uterus is markedly controlled by the activity of the corpus luteum. We were concerned therefore in ascertaining whether the association of ascorbic acid with the corpus luteum itself might not also be reflected in the uterus upon which corpus luteum secretion exerts such a marked influence. It has been demonstrated (Pincus and Kirsch, 1936; Pincus, 1936) that the growth of the blastocyst is probably not due to the direct stimulation of the corpus luteum hormone, but to a substance or special condition produced within the uterus by the action of progestin upon the uterine tissue. Conceivably ascorbic acid might be the substance produced or at least a factor in the special condition necessary for blastocyst growth and implantation. Accordingly we have measured the ascorbic acid concentration in the corpus luteum, the rest of the ovary, the uterus, and in the urine at various stages of pregnancy from the 1st to the 28th day of gestation. Pregnancy in the rabbit lasts from 30 to 32 days, so we have covered practically its whole course.

It may be stated at once that we have found a definite indication of a correlation of uterine ascorbic acid concentration with specific pregnancy

[1] This investigation was aided by a grant from the Josiah Macy Jr. Foundation.

stages, but our data show especially a remarkable correlation between ascorbic acid concentration and corpus luteum growth.

METHODS. Measurements on tissues of the reproductive tract were made at the times stated in the table. The animals were ordinarily killed by ether anesthesia, the tissues removed and weighed in the extraction fluid. The corpora lutea were easily shelled out from the surrounding tissue and carefully trimmed of any adherent non-luteal tissue with a pair of fine iris scissors. Ascorbic acid determinations were made by titration with 2–6 dichlorophenol-indophenol (Tillmans, Hirsch and Hirsch, 1932; Bessey and King, 1933; Harris and Ray, 1933; Birch, Harris and Ray, 1933). The dye was standardized with crystalline ascorbic acid.[2] The extraction fluid consists of 2 parts 8 per cent trichloracetic acid, 1 part metaphosphoric acid, and 1 part distilled water.[3] After weighing, the tissues are ground in a mortar with acid washed sand using successive portions of the extraction fluid, usually in three portions of about 3.3 cc. each. Titration with the dye is done as quickly as is consistent with accuracy, and the conditions are so arranged that not more than 2 cc. nor less than 1 cc. of the extract are used to give the end point.

The urines were collected in acid solution to prevent ascorbic acid oxidation but several collections made without the addition of acid showed only a slight loss of the vitamin. Urines were extracted and titrated in the the same manner as the tissues.

RESULTS. The data on the tissue extracts are presented in table 1. We shall first consider the various tissues separately.

Corpus luteum. The corpora lutea of the rabbit grow regularly up to the 28th day of pregnancy (662 hours after ovulation). The growth curve is presented in figure 1. The growth rate is extremely rapid up to the 3rd day post coitum (62 hours after ovulation) and then declines. This is illustrated in figure 2 in which the open circles represent Δ weight/Δ time taken from the growth data. In figure 2 also are presented the data on ascorbic acid concentrations in the corpora lutea over the whole growth period. The ascorbic acid concentration is at a maximum on the 3rd day also. It will be noted that the changes in growth rate of the corpora lutea consistently lag behind the changes in ascorbic acid concentration except in the latter part of pregnancy where they practically coincide. This implies that increases and declines in ascorbic acid concentration are *followed* by corresponding increases and declines in rate of corpus luteum

[2] Merck & Co. and Hoffman-La Roche were kind enough to supply us with crystalline ascorbic acid.

[3] We are indebted to Dr. C. G. King for suggesting the use of metaphosphoric acid in the extraction solution. The acid is beneficial in avoiding secondary atmospheric oxidation and oxidation by the CCl_3COOH itself. It does not interfere with the titration.

growth. The simple statement that the corpora lutea have a high ascorbic acid content does not, according to these data, suffice. In the later stages of pregnancy the ascorbic acid content is in fact below that of the whole ovary of unmated females and slightly below the content of the non-luteal portion of the same ovaries. The relation of ascorbic acid concentration to the growth phase of the corpus luteum is the significant correlation. This is roughly apparent in Biskind and Glick's (1936) data where maximum vitamin C content is reported for earliest formed (up to 6th day) corpora lutea in non-pregnant cows.

Non-luteal tissue of the ovary. These data are presented graphically in figure 3. Here too, but not so obviously, and certainly not to the same

TABLE 1

The ascorbic acid concentrations in various tissues of the rabbit during oestrus and pregnancy

Concentrations in milligrams of ascorbic acid per gram of tissue

NUMBER OF ANIMALS	TIME AFTER OVULATION	WEIGHT OF A SINGLE CORPUS LUTEUM	ASCORBIC ACID CONCENTRATION IN CORPORA LUTEA	ASCORBIC ACID CONCENTRATION IN THE OVARY WITHOUT THE CORPORA LUTEA	ASCORBIC ACID CONCENTRATION IN THE UTERUS	ASCORBIC ACID CONCENTRATION IN BODY MUSCLE
	hours	*mgm.*				
4*	0			0.302	0.185	0.011
5	8	0.54	1.415	0.364	0.117	0.012
4	62	4.14	2.745	0.303	0.152	0.010
1	89	7.02	1.275	0.438		
2	134	11.31	0.541	0.350	0.218	0.011
2	206	15.15	0.343	0.246	0.095	
2	278	18.05	0.293	0.267	0.119	
4	422	19.60	0.207	0.250	0.109	
2	662	20.85	0.185	0.245	0.072	

* Unmated females on heat.

extent, there is a rise in ascorbic acid concentration followed by a fall. The maximum occurs at the 4th day post coitum, with a sharp decline followed by almost no change from the 9th to 28th days. In the rabbit ovary there is a rather large proliferation of new follicles during the first few days of pregnancy or pseudopregnancy. Ovulation from a new set of follicles can in fact be induced by pituitary hormone injection on the 4th day (Pincus, 1936). The coincidence of rise in ascorbic acid content and this new and rapid follicle proliferation is not necessarily a causal correlation.

Uterus. The uterus shows a small but definite rise in ascorbic acid concentration during the first six days of pregnancy (fig. 3). Unfortunately we have no data for the uterus on the 4th or 5th days so the maxi-

mum appears to occur on the 6th day. From the 9th day onward there is at most a slight decline. This maximum coincides with the period of increased acidity found by Hall (1936) in the uteri of rats and mice.

Urine. Data on urinary ascorbic acid are given for four animals in table 2. Collections of urine were ordinarily made every 24 hours but the data are presented as milligrams excreted for 48 hours since more comparable volumes of urine are excreted over the longer period. The animals designated as A were mature females of an inbred strain, the animal X_1 was from another strain. The mean ascorbic acid excretion during pseudopregnancy in A_2 was significantly higher than during the periods of heat. During the last 20 days of pregnancy in rabbit X_1 the mean ascorbic acid excretion was higher than in the post-partum period but not significantly so on a statistical basis. Also the mean values for rabbits A_1 and A_4 are on the average less than the pseudopregnancy mean for A_2 but just

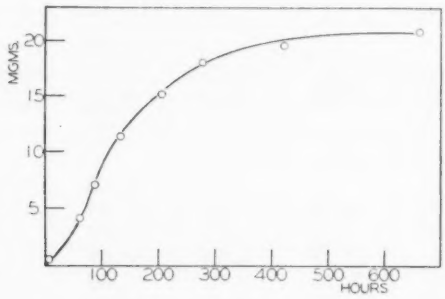

Fig. 1. The growth of the corpora lutea in the rabbit. Abscissa: time in hours after ovulation. Ordinate: mean weight of a single corpus luteum in milligrams.

on the level of significance. The indications are that ascorbic acid excretion increases during pregnancy, particularly during the early stages. Thus the mean value for the first six days of pseudopregnancy in rabbit A is 50 per cent higher than the mean for the last eight days.

Discussion. In the corpora lutea, the non-luteal portion of the ovary, and in the uterus, there is an unquestionable increase in ascorbic acid concentration during the first six days of pregnancy. In the uterus and ovaries where the change is of lesser magnitude the differences are statistically significant. Thus in the non-luteal portion of the ovaries the mean ascorbic acid concentration for all the determinations of days 1 to 6 is 0.349 mgm. per gram $\pm$ 0.0150 and the mean for days 9 to 28 is 0.249 $\pm$ 0.0062. The difference is 0.100 which is over six times the probable error of the difference $\pm$ 0.0162. Similarly for the uterus the values for the two periods are 0.150 $\pm$ 0.0123 and 0.100 $\pm$ 0.0044 with a difference of almost

four times its probable error, *i.e.*, 0.050 ± 0.0130. The determinations on body muscle ascorbic acid for these periods show no significant changes (see table 1). The first six days of pregnancy represent the preimplantation period. In the rabbit the ova are implanted on the 7th day. During this period the uterus is actively being prepared for the nidation of the ovum and during days 3 to 7 it is definitely controlling the growth of the uterine blastocysts (Pincus, 1936). The uterine proliferation that occurs preparatory to nidation is controlled by the secretion of the corpus luteum (Allen and Corner, 1929). The suggestion appears, therefore, that the corpus luteum hormone may be concerned in the production of ascorbic acid particularly in those tissues where it is active. Bourne (1935b) has

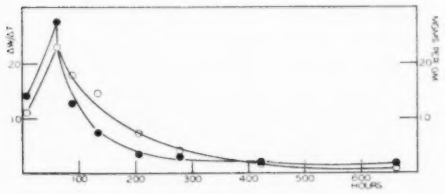

Fig. 2

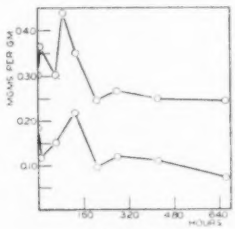

Fig. 3

Fig. 2. A comparison of the changes in ascorbic acid content of the corpora lutea and their rate of growth. Open circles = Δ weight/Δ time of corpus luteum. Closed circles = ascorbic acid concentrations. Abscissa: time in hours after ovulation. Ordinates: ascorbic acid concentrations in milligrams per gram; Δ weight/Δ time adjusted to approximately the same scale.

Fig. 3. Changes during pregnancy in ascorbic acid concentration in the uterus and non-luteal portion of the ovary of the rabbit. Upper curve = data for the non-luteal portion of the ovary. Lower curve = data for the uterus. The 0-hour point is for animals in heat. Abscissa: time in hours after ovulation. Ordinate: ascorbic acid concentration in milligrams per gram.

prevented the appearance of symptoms of scurvy in guinea pigs on a scorbutic diet by the daily injection of 50 rat units of Antuitrin-S. The Antuitrin-S injections cause ovarian luteinization; he states furthermore that pregnant guinea pigs with corpora lutea in their ovaries are protected from the effects of a vitamin-C-free diet. Kramer, Harmon and Brill (1933), on the other hand, report definite effects of a scorbutic diet on pregnant guinea pigs, *e.g.*, embryo death and abortion. Their data do show poorly developed corpora lutea in scorbutic animals and even failure of true corpus luteum development. Our data on the corpora lutea show that increases and decreases in ascorbic acid content precede corresponding changes in growth rate, the implication being that vitamin C controls corpus luteum growth. We can make no deductions concerning hormone

TABLE 2

Urinary ascorbic acid in unmated, pseudopregnant and pregnant rabbit does

Milligrams of ascorbic acid per 48 hours

ANIMAL NUMBER	CONDITION	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	MEANS	
																					Non-pregnant	During pseudo-pregnancy or pregnancy
A_1	Unmated throughout	2.100	0.590	0.623	1.036	1.060		1.191	0.798	0.906	1.228	0.695									1.022 ±0.085	
A_2	Pseudo-pregnant period†	1.320	0.699	1.087	0.702	*1.356*	*1.100*	*2.990*	*1.144*	*0.998*	*1.190*	*1.180*	0.785	1.176	*	0.758	0.484	0.739			0.861 ±0.066	1.423 ±0.155
A_4	Unmated throughout	0.578		1.280	1.145	1.425	0.676	0.544	0.916	*	0.590	0.490									0.849 ±0.091	
X_1	Last 20 days of pregnancy and post-partum‡	*4.74*		*4.780*	*2.157*	*1.096*	*3.242*	*2.016*	*1.126*	*2.650*	*1.628*	1.451	2.106	1.354	2.345	2.288	*	1.008	1.472	1.028	1.632 ±0.147	2.604 ±0.312

* 10 day interval with no determinations.

† The italicized data are for the period of pseudopregnancy.

‡ The italicized data are for the pregnant period.

function, since we do not know the hormone content nor rate of hormone production of the corpora lutea at the various stages. The effects of Antuitrin-S described by Bourne may not necessarily be due to the luteinization but to a general stimulation of proliferative activity in the ovary and perhaps in other tissues. Our data do show that vitamin C concentrations are high where active tissue proliferation is occurring. This is certainly true of the corpora lutea and probably also of the uterus and non-luteal portion of the ovary, though the increases in the latter may be simply due to diffusion of vitamin from the corpora lutea into the neighboring tissue. Our data on urinary ascorbic acid are insufficient to permit the deduction of a correlation between the general organic activity occurring during pregnancy and vitamin-C economy. They suggest increased excretion during pregnancy and especially during the early period (note the marked rise in animal A_2 for days 5 and 6 of pseudopregnancy).

Mosony (1936) has shown that the injection of oestrogenic compounds causes a reduction in the ascorbic acid concentration of the liver. The liver is certainly a site of oestrogenic hormone destruction (Zondek, 1934) and it is possible that this inactivation should involve a reduction of the hormones. On this basis corpus luteum hormone might invoke an increased concentration of ascorbic acid in the tissue which it affects and so inactivate by reduction the oestrogens which oppose progestin action.

SUMMARY

The ascorbic acid concentration of ovarian and uterine tissues of the rabbit has been determined in females in heat and during the various stages of pregnancy. In the corpora lutea the concentration rises to a maximum at the 3rd day of pregnancy and then declines. This is closely correlated with the rate of growth of the corpora lutea. The ascorbic acid concentration appears to increase more rapidly and, in the decreasing phase, declines more rapidly than the growth rate. The non-luteal portion of the ovary and the uterus also exhibit an increased ascorbic acid concentration during the first six days of pregnancy. The coincidence of these increases in vitamin C content with the preimplantation period is noted. No such increase occurs in body muscle which was examined as a control tissue.

Urinary ascorbic acid appears to be higher in pregnant than in non-pregnant animals, especially during early pregnancy.

Very good material for paper on kidney.

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ENHANCEMENT OF MUSCLE CONTRACTION AFTER TETANUS[1]

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Received for publication December 28, 1936

While the course of fatigue in the isolated nerve-muscle preparation is well known, certain effects concerned with fatigue apparently have been previously neglected. These effects would seem to cast light on the manner of excitation of skeletal muscle by its motor nerve. It has been observed, on indirectly stimulated nerve-muscle preparations, that a 5 to 10 second period of change from a low rate of stimulation to a high rate of stimulation produces, on return to the original low rate, an increase in the height of contractions (Guttman, Horton and Wilber, 1936). This paper is a more detailed report on studies on this "enhancement" phenomenon with special reference to its relation to the rate of stimulation, to the state of fatigue of the preparation and to the effects of various drugs.

PROCEDURE. Sciatic-gastrocnemius preparations of the frog were stimulated with constant voltage break shocks which were initially maximal. The free-weighed lever carried 20 grams at 0.8 the muscle distance and magnified 8 times. The lower rates of stimulation were about 1 per second and higher rates (tetanus) ranged from 10 to 30 per second. Tetanus for 5 seconds preceded and followed by 1 per second shocks we call the tetanus cycle. The contractions after tetanus are considered enhanced if they are greater in amplitude than the last contraction preceding tetanus, so that absolute enhancement is the increment, while per cent enhancement is the increment in per cent of the last contraction before tetanus and relative enhancement is the ratio of the two amplitudes.

Enhancement in normal preparation. A. *State of fatigue.* 1. *Tetanus in late fatigue.* Tetanus delayed until the height of contraction had become 50 per cent or less of the initial maximum height produced, on return to the original low rate, contractions as high as (or even higher than) the initial maximum height. The enhancement sometimes lasted as long as 60 seconds after cessation of tetanus. The tetanus cycle could be repeated at half minute intervals, for as many as 5 to 10 times with resultant enhance-

[1] This work was made possible by grants from the Sarah Manning Sage and Solon P. Sackett Research Funds.

ments. Figure 1B is a typical record. Figure 6 shows the enhancement phenomenon at another stage of late fatigue. A muscle which had not been previously tetanised and which gave no recorded contraction could, after tetanus, give marked contractions; *i.e.*, infinite enhancement for as many as 5 periods of tetanus.

2. *Tetanus in early fatigue.* Tetanus, applied when the amplitude of the contractions was 95 per cent to 50 per cent of the initial maximum height, caused a marked enhancement (figs. 2, 5B, 7). Repetitions of cycles of tetanus showed an increased percentage enhancement until it finally became infinite. (See figs. 1B, 3B, 7.) With this repetition beginning in early fatigue, in late fatigue neither as great nor as lasting infinite enhancement, nor enhancement for as many cycles, could be obtained as compared with a preparation in which tetanus had been delayed until late fatigue. (See fig. 6.)

3. *Tetanus in non-fatigued muscle.* Tetanus (10 per sec.) applied before the contractions began to decrease produced no enhancement but with a higher rate (20–30 per sec.) a slight but very enduring enhancement occurred in winter frogs. (Figs. 3, 5A, 6A, 10A.) In summer frogs this treatment produced tremendous contracture (usually permanent), with sometimes decreased but still large contractions (fig. 4).

Frequency of the tetanising current. The size and persistence of enhancement increased with the frequency of tetanus up to 20 per second, beyond which (up to 30 per sec.) the increase was slight. See figure 3A for 20 as compared with 30 (fig. 3B) and figure 9 for 10 and 20 per second.

The effect of the length of tetanus. To determine the period of tetanus which produced optimal enhancement, tetanising frequencies (20 per sec.) were applied for a period range of 1 to 20 seconds. Tetanus for one second caused very slight, and for 2 seconds distinct, enhancement; for 5 seconds, an enhancement still greater in height and duration; 10 to 20 seconds of tetanus only slightly greater enhancement than for a 5 second period. Periods longer than 5 seconds produced a brief contracture, which followed tetanus immediately in early and late fatigue but which was delayed in middle fatigue.

Plate I. Fig. 1 A. Stimulation 65 per min. Rest periods 15 sec. 1 B. Continuation of A. Tetanus frequency 20 per sec. Rest periods after tetanus 15 to 2 sec.

Fig. 2 A and B. Stimulation 60 per min. Tetanus 20 per sec. Rest period after tetanus 5 sec.

Fig. 3 A. 65 per min. and 20 per sec. 3 B. 65 per min. and 30 per sec.

Fig. 4. Summer frog. 65 per min. and 30 per sec.

Fig. 5 A and B. 70 per min. and 20 per sec.

Fig. 6 A and B. 60 per min. and 20 per sec. Rest after tetanus 5 sec.

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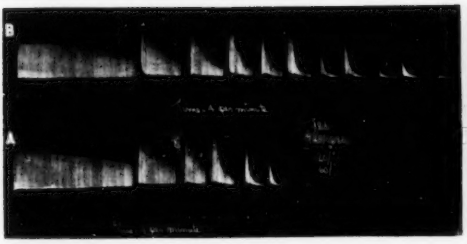

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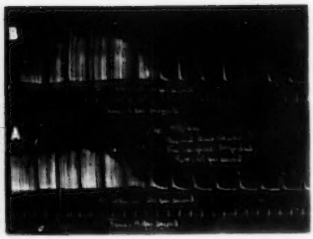

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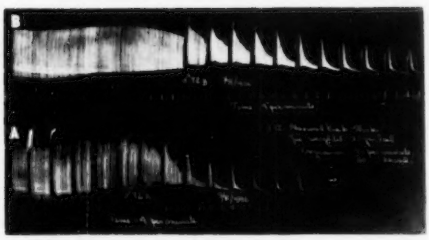

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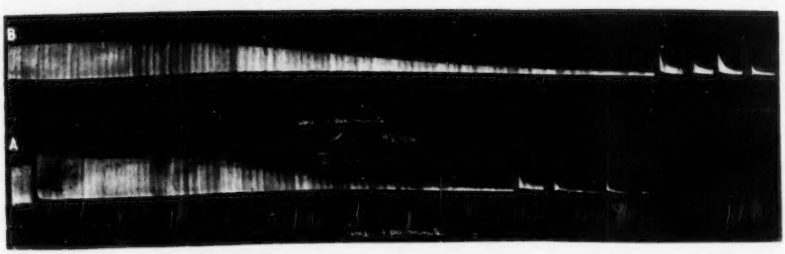

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PLATE 1
Figs. 1 to 6

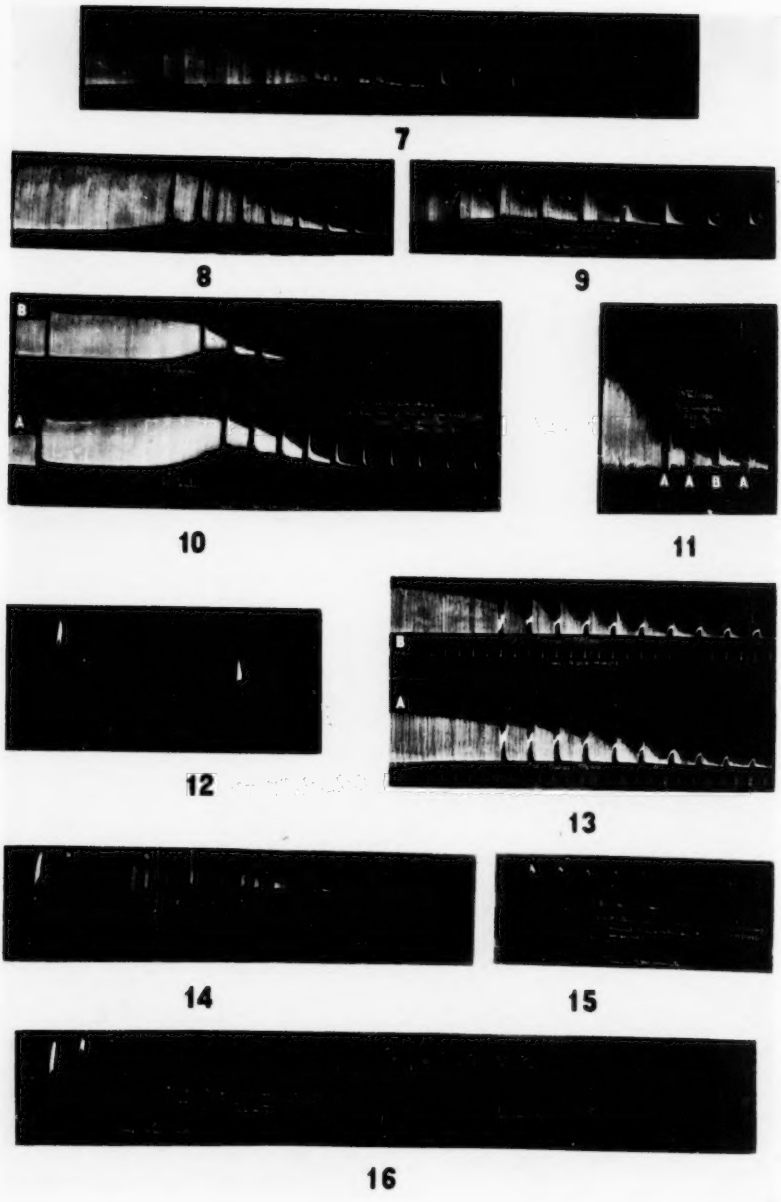

PLATE 2
Figs. 7 to 16

Suppression after tetanus. In some cases suppression has been observed especially with submaximal 1 per second stimuli following maximal tetanic shocks. The suppression ranges all the way from complete lack of response after tetanus, followed by recovery to normal, to a slightly decreased enhancement followed by full enhancement. This may also be caused by a long period of tetanus and by a very high frequency (130 per sec.) for 5 seconds.

Rest versus tetanus. The increase in contraction after rest is never as great as after tetanus nor does it last as long (figs. 1 and 7). The increase after a 5 second rest is greater in early fatigue than later, there being no increase in late fatigue. After tetanus, on the other hand, the enhancement grows greater with the progress of fatigue.

Effect of rest after tetanus. With either rest after tetanus or stimulation at 1 per second for the same length of time, the enhancement decayed at about the same rate. Thus, the height of the sixth contraction after tetanus was approximately the same as that of the first contraction after 5 seconds of rest after tetanus. See figure 1B for rest periods of 15, 10, 5 and 2 seconds, and figures 2 and 6 for 5 second periods.

Enhancement in voluntary contraction. An attempt was made to observe the enhancement phenomenon in the voluntary contraction of human muscle, as recorded by Mosso's ergograph. The subjects (medical students) lifted a 3.2 kgm. weight by flexion of the middle finger at a rate of 1 per second. When evidence of fatigue showed on the record, the weight was maintained as high as possible for 5 seconds. This was assumed to be equivalent to a period of tetanus, since there was a larger number of stimuli conducted per second than with lifting the weight once per second. Definite enhancement occurred which was as great, and in some cases greater, than that after a similar period of rest (fig. 11).

That central fatigue is an important factor is shown by the decreasing contraction during the maintenance of tension. It has been found in

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Plate II. Fig. 7. 60 per min. Tetanus 20 per sec. Rest period 5 sec.

Fig. 8. Effect of eserine 65 per min., 20 per sec.

Fig. 9 A. 60 per min. B. 10 per sec. C. 20 per sec.

Fig. 10 A. Control preparation 65 per min., 20 per sec. B. Atropine preparation 65 per min., 20 per sec.

Fig. 11. Mosso's ergograph record, middle finger, free-weighted 3.2 kgm., 60 per min. A. Maintained tension. B. Rest.

Fig. 12. Monoiodoacetate preparation, 30 per min., 10 per sec.

Fig. 13 A. Control preparation 65 per min., 10 per sec. B. Cocaine preparation 65 per min., 10 per sec.

Fig. 14. Lactic acid preparation, 60 per min., 10 per sec.

Fig. 15. Curare preparation. (Direct stimulation.) Sixty per min., 10 per sec.

Fig. 16. Control for lactic acid (fig. 14), 60 per min., 10 per sec.

isolated preparations that decreasing contraction during tetanus results in an enhancement less than that after a tetanus in which the contraction did not decrease.

Localisation of phenomenon. As the phenomenon appeared under the conditions stated above, it is apparent that enhancement may take place in any portion of the motor unit; namely, in the muscle fiber, in the myoneural junction, or in the motor nerve at the point of stimulation. Hence a series of experiments was performed on directly excited muscle. Tetanus (10 per sec.) in late fatigue for 5 seconds produced enhancement in some muscles and not in others. Enhancement under such conditions was taken as an indication of myoneural stimulation since it had all the previously mentioned characteristics. To ensure direct stimulation, preparations were curarised until the muscle just ceased to respond to indirect stimulation so that the myoneural junctions were paralysed and the autonomic fibers presumably were not. Simultaneous tetanising of the sciatic and direct stimulation (1 per sec.) of the muscle produced no enhancement, nor did direct tetanisation alone. Therefore, when enhancement did appear in the uncurarised preparations, stimuli were probably going through some of the myoneural junctions or even nerve fibers rather than stimulating the muscle fibers directly. Consequently, the muscle fiber is not the locus of enhancement.

Experiments were performed by Guttman and Comden on frogs, using two pairs of electrodes on the sciatic nerve of the gastrocnemius (intact circulation). One pair was for tetanising shocks and the other for stimulation at 1 per second. In some experiments the tetanising electrodes were nearer to, and in others farther from, the muscle than the low frequency electrodes.

The enhancement phenomenon was unaltered by either procedure. This shows that there is no change, caused by tetanus, in the local excitatory state of the nerve, in the vicinity of the electrodes, to which the enhancement phenomenon might be ascribed.

Eserine and atropine series. Eserine. Preparations from frogs injected with 0.5 mgm. of eserine salicylate showed only a very slight enhancement following a tetanus of conspicuously subnormal height (see fig. 8). Fatigue appeared earlier and contracture was often greater.

Atropine. Preparations soaked in atropine (1:1000 Ringer's) showed an enhancement less than normal which decayed rapidly after a tetanus which was characterized by a fall instead of a rise. Contracture was less and the general fatigue was greater than in the normal control (see fig. 10B).

Cocaine and ergotoxine series. Cocaine. Preparations soaked in cocaine hydrochloride (1:20,000 Ringer's) showed a tetanus markedly clonic and below normal in height while the initial twitch after tetanus was about the

same in height as the control. However, the decay of enhancement was much more rapid with cocaine, so that in a series of cycles the enhancement was greater in the treated preparations. There was little or no contracture with cocaine. (See fig. 13.)

Ergotoxine. The enhancement phenomenon was unaltered in preparations from frogs injected with 2 to 4 cc. of ergotoxine (1:2000 Ringer's) or in preparations soaked in this solution.

Lactic acid and iodoacetic acid series. Lactic acid. Preparations soaked for 10 minutes in 1 per cent lactic acid Ringer's solution exhibited less enhancement than normal but this might be ascribed to the more rapid general fatigue of the muscle fibers. The shape of the tetanus was unaltered and contracture during stimulation was the same as in the controls. See figures 14 for lactic acid and 16 for the control.

Iodoacetate. Preparations from frogs injected with 20 mgm. of sodium monoiodoacetate showed no enhancement after tetanus given either before or after the onset of fatigue, but instead the tetanus markedly increased the fatigue (see fig. 12).

SUMMARY AND DISCUSSION. I.A. Apparently there is a reserve of energy in the motor unit which is not called forth by 1 per second stimulation. But, by a mechanism which is brought into action by a short interjected period of higher frequency stimulation, this reserve is released, under certain conditions, for use at 1 per second. The availability of this mechanism is limited and, if frequently called upon early in fatigue, cannot be brought into action later. (See figs. 5 and 6.)

B. This reserve energy on being released disappears after tetanus as a function of time and is not used up by contraction as shown by the results of rest after tetanus. This disappearance may be due to the destructive action of an enzyme or to some rearrangement of the chemical mechanism involved.

C. Rest without tetanus is never as effective as tetanus in increasing the subsequent muscular response. That the recovery involves a different mechanism in the two cases is also evidenced by the following: 1, the characteristic recoveries are entirely different in all stages of fatigue; 2, while a previous period of tetanus diminishes the enhancement after subsequent periods of tetanus, previous periods of rest do not; 3, combinations of tetanus and rest do not produce a summated enhancement.

II.A.1. The earlier in fatigue, the greater is the frequency necessary to produce enhancement. In unfatigued preparations a frequency of 20 to 30 per second was necessary to elicit enhanced responses while late in fatigue 2 per second might be sufficient.

2. At a given state of fatigue with a given period of tetanus, the higher the frequency (up to 30 per sec. at least), the greater is the resulting enhancement both in size and duration.

B.1. The earlier in fatigue, the longer is the period necessary, for a given frequency of tetanus, to produce an enhancement.

2. At a given state of fatigue with a given frequency of tetanus, the longer the period of tetanus, the greater is the resulting enhancement both in size and duration, provided that the tetanus is not so long (over 20 sec.) as to show marked fatigue in itself.

C. As a result of the previous statements it would seem that the product of the duration and the frequency; *i.e.*, the total number of stimuli, is the controlling factor in determining the size and duration of the enhancement. The progress of fatigue during tetanus tends to obscure this comparison as made experimentally.

III. It was noticed in those experiments in which tetanus height was not maintained, that the resulting enhancement was less than would otherwise have been expected.

IV. In voluntary contraction of the human subject enhancement was observed after maintenance of tension in spite of the depressing factor of central fatigue.

V.A. Since the myoneural junction is known to fatigue before the motor nerve or its skeletal muscle, it seems probable that the recovery from fatigue due to tetanus may be also located at the myoneural junction. The experiments with directly stimulated curarised muscle, which showed no enhancement, indicate that the phenomenon does not result from a change in the muscle due to tetanic contraction.

B. Waller (1897) showed that there was an apparent increase in the action potentials of a nerve subjected to 7 seconds of tetanic stimulation once a minute (corresponding to our single stimuli once per second) after 5 minutes of tetanic stimulation (corresponding to our 5 seconds) had intervened.

Davis and Davis (1932) found that an indirectly stimulated muscle (cat's soleus), after being fatigued by 30 per second stimulation, recovers both as to action potentials and amount of tension on being stimulated at a lower rate (15 per sec.).

Gasser and Grundfest (1936) show that there is an actual increase in the action potential spike of the nerve after tetanus as compared with that before tetanus. Their low rate of stimulation was once in 2 seconds at one-fifth maximal strength, while the tetanus (not lower than 250 per sec.) was maximal for a period of 10 seconds. They believe that these variations are due to change in the number of nerve fibers conducting.

Therefore the possibility that the enhancement phenomenon may be due, in part at least, to the nerve cannot be excluded. The experiments of Guttman and Comden indicate that the change, if any, in the excitatory state of the nerve is not confined to the region of the tetanising electrodes. This holds only for a tetanus of approximately maximal strength, but

whether it is true for tetanus of supermaximal strength, after which suppression of contractions is observed, is not known.

C. Other experimenters (Boyd, 1931; Boyd and Brosnan, 1936; and Lubinska, 1935) using a technique similar to ours have overcome myoneural block produced by curare or magnesium salts. However, they do not report the phenomenon in the normal preparation. Boyd was unable to overcome curare block in the frog but succeeded in the cat.

VI.A. Dale and his co-workers believe that acetylcholine, liberated by the motor nerve, is the mediator in the myoneural junction and initiates the muscular contraction (Brown, Dale and Feldberg, 1936).

Lapicque (1936) asserts that acetylcholine by itself at the myoneural junction does not initiate muscular contraction but merely assures the spread of the electrical excitation from the nerve into the muscular substance by intensifying the impulse. Such a theory would seem to be applicable to our results, since it allows the accumulation of acetylcholine without maintained contraction, while Dale's experiments would seem to indicate that as long as acetylcholine is present in the myoneural junction the muscle maintains contraction. One interpretation of our experiments might be that the accumulation of acetylcholine, after tetanus, could render more muscle fibers excitable by the nerve impulse than before.

B. The effect of eserine in abolishing enhancement can be explained in part by the high concentrations used. Eserine in concentrations greater than that needed for the maximum effect abolishes the normal excitatory effect of acetylcholine in the superior cervical ganglion, by bringing about an excess of acetylcholine (Brown and Feldberg, 1936).

Heirman (1936) has noted the depressing and then the blocking of the myoneural junctions of the frog's gastrocnemius by large amounts of acetylcholine. It seems strange that in our experiments with eserine we did not observe a depression and then a rise of muscular response after tetanus, but instead the responses steadily declined. Thus it would seem, on the basis of the acetylcholine hypothesis, that the acetylcholine liberated by tetanus is more affected by eserine than that liberated at a lower rate of stimulation. However, we did observe this depression followed by a rise in our suppression experiments in which the effects of eserine in high concentrations were absent.

C. Since atropine decreases tetanus height, contracture and enhancement after tetanus, this might also be taken as an indication that acetylcholine participates in the production of enhancement.

VII.A. Orbeli (1925) found that stimulation of the postganglionic sympathetic fibers to skeletal muscle caused increase of its contractions if it were fatigued. Corkill and Tiegs (1933) found that perfusion of skeletal muscle with adrenalin gave similar results. With both perfusion and

sympathetic stimulation there is a relatively long latent period before the appearance of the increase, presumably due to the time necessary for diffusion from the blood vessels into the muscle. In contrast the enhancement phenomenon has a latent period of less than a second.

Unpublished observations of Guttman and Comden on sympathectomized frogs show that sympathectomy has no effect upon the enhancement phenomenon. Thus we conclude that enhancement is not due to an adrenergic substance set free by the sympathetic nerves. Still there exists the possibility of liberation of an adrenine-like substance at the myoneural junction.

B. Cocaine is known to potentiate the action of adrenine in some cases. It seemed to potentiate the first few contractions of the enhancement at the expense of the later contractions. Thus there exists the possibility that adrenine is concerned with enhancement. The lack of effect of ergotoxine on the phenomenon may be taken as an indication that the sympathin mechanism is not concerned here.

VIII. The effects of lactic acid may be interpreted as those of fatigue of the muscle fibers so that they cannot respond fully to the changes brought about by tetanus in the myoneural junction. Similarly, we may interpret the decreased height of contraction, after tetanus, of the muscle poisoned with iodoacetate, to the rapid depletion of the energy supply of the muscle by tetanus.

IX. The possible influence of K′ and other ions has not been considered here. See Gellhorn (1932) and Wilson and Wright (1936).

GENERAL CONCLUSIONS

We conclude that the locus of the enhancement phenomenon is the myoneural junction, although the possibility of participation by the nerve cannot be neglected. The accumulation of a chemical mediator in tetanus at the myoneural junction offers a reasonable explanation of our findings. The chemical mediator may be acetylcholine or adrenine.

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THE EFFECT OF PROTEIN DEFICIENCY ON THE COURSE OF PREGNANCY

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Received for publication December 29, 1936

When the minimum level of nitrogen excretion is reached on a N-free diet, fed in quantities sufficient to meet the caloric requirements, the deposit[2] protein is said to be depleted and any nitrogen which is further excreted can be considered as a measure of the endogenous metabolism or the protein maintenance requirement.

Gestation superimposes a further drain on the nitrogen reserve, since the growth of the fetus, if it survives, requires a considerable quantity of nitrogen. The work presented here is an attempt to clarify the effects of maternal nitrogen starvation upon gestation and the developing fetus. Accordingly the general objectives were 1, to determine during how much of the gestation period rats can be maintained on a nitrogen-free diet and still produce offspring; 2, to determine what effect, if any, such specific nitrogen starvation has on the birth weight of the offspring, and 3, to measure both the absolute quantity of nitrogen in the young at birth and the quantity per unit body weight under the above experimental conditions.

EXPERIMENTAL. Albino rats of vigorous local stock were fed a commercial ration containing 18 per cent protein, while not on the experimental diets. The composition of the latter is given in table 1. Mating was controlled by vaginal smears and at various times in pregnancy the animals were transferred to individual metabolism cages and given the N-free diet *ad libitum*.

Of those which were placed on the diet between the 13th and 10th day before parturition as many failed to produce young as gave birth to young. No attempt was made to determine with precision the probability of the

[1] This work was done at the Samuel S. Fels Research Institute for Study in Prenatal and Postnatal Environment, and published with the permission of the director, Dr. L. W. Sontag.

[2] The terms deposit and reserve protein are not particularly definitive. The term reserve protein is used with the following connotation in this paper: the total quantity of protein an animal, in normal nutritive condition, may have available for the maintenance of life when given a "nitrogen-free" diet. This does not conflict with such studies as those of Addis, Poo and Lew (8).

outcome of the experiment for any given time of feeding the N-free diet. On the basis of data obtained, we can state that if the N-free diet is instituted on or before the 13th day preceding parturition, the fetus' chance for survival is slight; if it is instituted between the 10th and 13th day, it has approximately an even chance, and birth will probably ensue if the diet is not fed earlier than the 10th day before parturition.

From table 2 it can be seen that through such specific protein starvation the body weight and protein content of the young at birth are less than that of control animals receiving the stock ration. There was apparently also less nitrogen per unit body weight in the offspring of these animals. This nutritional impoverishment of the young is especially marked when the mother's time on the diet is extended beyond 8 days.[3]

The urine nitrogen excretion of 5 of the pregnant animals was determined and the figures are given in table 3. Many variables influence

TABLE 1

*Composition of experimental rations**

	GELATIN	N-FREE
	per cent	*per cent*
Gelatin	10.0	
Hydrogenated cottonseed oil†	15.0	15.0
Cornstarch	66.5	76.5
Agar	2.0	2.0
Salt mixture‡	4.5	4.5
Cod liver oil	2.0	2.0

* Supplemented daily with a yeast concentrate containing 5 mgm. of nitrogen.

† Crisco.

‡ Osborne and Mendel's as modified by Hawk and Oser (7).

the quantity excreted and only consistent trends are regarded as significant. The initial excretion and that coincident with birth was always high but between these periods there is a tendency toward constant excretion. This last characteristic is especially evident in the case of rat NF-8. The minimum excretion per 100 grams body weight before delivery compares favorably with the minimum excretion after delivery. The purpose of these preliminary urine analyses will be discussed more fully later.

The marked capacity of the maternal organism to supply nitrogen from its reserves is apparent from even a casual inspection of tables 2 and 3; but for purposes of contrast a few pregnant animals were given

[3] The young of rat number NF-4 (four-day experiment) were small, but this is undoubtedly due to the large litter. This variable can only be eliminated by increasing the number of experiments and comparing only litters of equal size.

TABLE 2

The birth weight and nitrogen content of the young when the mothers received a nitrogen-free, gelatin, or a stock ration

RAT NUMBER	NUMBER OF DAYS ON RATION	NUMBER IN LITTER	AVERAGE WEIGHT OF YOUNG	NITROGEN CONTENT OF YOUNG	NITROGEN PER GRAM BODY WEIGHT
			grams	*mgm.*	
NF-4*	2	7	5.14		
NF-3	3	7	4.63	69.0	14.9
NF-16	3	7	5.04	61.5	12.2
NF-4	4	9	4.58	52.9	11.6
NF-21	7	7	4.59	64.0	13.9
NF-9	7	7	4.60		
NF-4	8	7	4.86	66.5	13.7
NF-11	9	6	4.83	58.8	12.2
NF-8	13	6	4.33	55.9	12.9
NF-5	11	7	4.28	57.0	13.3
NF-15	10	7	4.28	55.9	13.1
NF-21	10	4	4.25	51.7	12.2
NF-16	11	6		50.0	
NF-18	12	5	4.00	50.7	12.7
NF-13	13	6	4.16		
Average			4.54	57.8	13.0
G-2*	13	5	3.40	49.9	14.7
G-3	10	7	3.51	55.8	15.9
G-4	11	6	4.15	63.7	15.3
G-18	11	9	3.27	47.5	14.5
G-16	9	8	3.60	53.9	15.0
G-40	12	2	4.87	82.7	17.0
G-41	9	6	4.72	73.0	15.4
G-21	7	8	3.87	51.5	13.3
Average			3.92	59.7	15.1
S-13*		7	5.58	87.8†	15.7
S-6		7	5.10	76.7	15.0
S-8		7	5.44	80.7	14.8
S-2		6	5.55	82.7	14.9
S-17		6	5.00	76.1	15.2
S-1		3	5.87	90.3	15.4
Average			5.42	82.4	15.2

* Series NF received the nitrogen-free ration, series G, the gelatin ration, and series S, the stock ration.

† In the S series it was in most cases assumed that there is a direct proportionality between weight and nitrogen content of the young and only a few animals of each litter were used for analysis. The nitrogen figure given for unit body weight is accordingly based only on the weight of the young taken for analysis and their N content.

an adequate ration containing 13.2 mgm. of nitrogen per gram. One of these animals weighed 186 grams at the beginning of pregnancy and stored on the average (disregarding fecal loss) 103 mgm. of nitrogen daily. In considering the contrast between this typical animal and those on the N-free diet over a relatively long period of time, one can, in effect, credit most of the nitrogen content of the young (N-free diet) to the negative balance of the maternal tissues because most of the fetal tissue is formed in the last 7 days of pregnancy (1).

TABLE 3

Urine nitrogen excretion on nitrogen-free ration during pregnancy

Results expressed in milligrams per day*

DAYS BEFORE PARTURITION	RAT NUMBER				
	NF-11	NF-8	NF-15	NF-21	NF-16
13					
12		76.5 (46)			
11		76.5			
10		54.2 (35)	53.6 (34)	74.8 (53)	84.5 (56)
9	101.1 (55)†	54.2	53.6	74.8	84.5
8	101.1	50.9 (34)	53.6	50.8 (37)	50.9 (33)
7	67.5 (38)	50.9	41.6 (27)	50.8	50.9
6	67.5	49.2 (33)	41.6	25.7 (19)	42.7 (28)
5	51.9 (29)	49.2	43.7 (28)	25.7	42.7
4	51.9	50.3 (34)	43.7	31.6 (23)	36.8 (24)
3	56.1 (31)	50.3	42.0 (27)	31.6	36.8
2	56.1	51.1 (33)	42.0	31.5 (22)	67.3 (44)
1	80.5 (58)	51.1	46.0 (37)	31.5	67.3
0	80.5	101.1 (90)	46.0	52.9 (47)	59.8 (40)
−1	51.2 (38)	101.1	35.5 (30)	52.9	59.8
−2	51.2		35.5	24.2 (22)	35.8 (31)
−3	32.9 (26)		25.8 (23)	24.2	35.8
−4	32.9		25.8	20.5 (19)	30.1 (28)
−5			23.2 (21)	20.5	30.1
−6			23.2	22.2 (21)	
−7				22.2	

* 48-hour collections were analyzed.

† The figure in parentheses represents the number of milligrams of nitrogen excreted per 100 grams body weight.

Rats on the N-free diet gradually lost weight until the last few days before parturition when the loss was arrested and was followed, in some cases, by a slight gain. When the animals went to term, the young were always born alive and no changes in the length of the gestation period were noted.

In a comparable experiment when the ration contained 10 per cent gelatin (table 2) as the source of protein, the behavior of the animals

was similar. When the diet was instituted early in pregnancy they failed to give birth to young, and when the young were carried to term they weighed less and contained less nitrogen than those of the control rats. There was also a tendency for them to contain less nitrogen per unit body weight, but in view of the small difference the unusual inequality of the litter size must be considered. It seemed probable that less striking results would be obtained than with the N-free diet, because it is reasonable to suppose that a small but significant quantity of essential amino acids derived from the interstices of the maternal tissues would be available for supplementing the deficiency of the gelatin. The work of Mezincesco (2) suggests that non-essential amino acids can in part cover endogenous nitrogen requirements. Furthermore variable and sometimes high biological values have been reported for gelatin (3). If any benefit was derived from the gelatin in these experiments, it must have been small (table 2). In this connection, the earlier work of Hart, Nelson and Pitz (4) is also of interest. Although their rats were given what they called a lysine deficient diet which they did *not* regard as fully adequate for maintenance, the young were born regularly. In view of the small requirements of lysine for maintenance, if it is needed at all for that purpose (5), it seems probable that the yeast (0.5 to 2 per cent of the ration) proteins in their ration supplied enough lysine to carry them through the gestation period. It is now known that yeast proteins contain considerable quantities of lysine (6).

Discussion. In addition to measuring the quantity of nitrogen lost in the urine, the figures in table 3 were secured for the purpose of ascertaining whether such data can be of any value in studying the relative requirements of nitrogen for growth and maintenance. The results with rat NF-8, for example, indicate that the transfer of the amino acids from the maternal tissues to the fetuses is accomplished without loss of nitrogen. If the growing fetuses required an assortment much different from that yielded by the sacrifice of the maternal protein, a comparatively large increase in the excretion of urine nitrogen should result as a consequence of wastage representing the amino acids not required by the fetuses for growth. The toll exacted by the young must have deprived the maternal organism of reserve protein which could otherwise have been used for endogenous metabolism. If the protein supply for endogenous metabolism were not depleted by the young, these mothers should survive as long on a N-free diet as any non-pregnant animal placed on the same diet. The survival time was not determined, but the mothers were in poor condition after birth and probably would not have lived as long on the N-free diet as non-pregnant rats have been known to live on similar diets. Although these results (table 3) show that data on rats leave much to be desired, they indicate that this experimental technique can be

used to secure valuable information on nitrogen metabolism. If a more suitable animal than the rat could be secured, namely, one in which pregnancy can be maintained longer on a nitrogen-free diet, the conditions would be even more favorable for studying the relationship between maintenance and growth requirements. The fetuses are essentially measurable growth units superimposed and intimately associated with the maternal maintenance unit.

SUMMARY

Pregnant albino rats can be given a nitrogen-free diet as early as 13 days before term and still bear living young. If they are fed such a diet earlier than 8 days before delivery there is marked intrauterine nutritional impoverishment of the young. They weigh less at birth, and contain less total nitrogen and less nitrogen per unit body weight than the young of adequately fed control animals. Experimental results with a diet containing 10 per cent gelatin as the source of protein were similar to those on the N-free diet. It is questionable whether the young of mothers on the gelatin diet contained less nitrogen per unit body weight than the controls.

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THE RELATION OF THE SIZE OF THE MEAL TO THE EMPTYING TIME OF THE HUMAN STOMACH[1]

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Received for publication January 8, 1937

It is logical to assume and it is generally understood that it takes longer for a large meal to leave the stomach than it does for a smaller one. While a great deal of work has been reported on the emptying time of the stomach no carefully controlled work could be found in the literature which showed the actual relation of the amount of food ingested to the emptying time of the stomach.

Since many different sized meals have been used by various investigators, a quantitative study of this factor seemed desirable as it is often impossible to compare or evaluate results. Moreover, it seemed of interest to determine how much a large meal would delay gastric emptying time and in this way delay the onset of hunger contractions, for the latter do not start until the stomach is practically empty. Advantage was taken of the fact that material and data were at hand to make this study a comparatively simple one (1).

METHOD. Normal human subjects were used in this work. The normal gastric emptying time was determined in six healthy young male subjects. The standard meal used was the same as was reported earlier (2). Fifteen grams of Quaker Farina were boiled in 350 cc. of water until the total volume equalled 200 cc. Fifty grams of barium sulphate were added so that the stomach could be seen fluoroscopically. One gram of sodium chloride was added to improve the taste of this mixture. The meal was eaten at 8:30 a.m. No food had been taken since the preceding evening.

A number of normal determinations were made for each individual and the average figure was used for the norm.

After these data had been obtained, the subjects were asked to eat a meal just twice the size of the standard meal, that is, this meal contained 30 grams of Farina instead of 15 grams. The gastric emptying time was again determined and the average figure was used.

The subjects were now given a meal three times the size of the standard meal or 45 grams of Farina. This type meal was eaten twice and the

[1] Aided by a Grant from the Committee on Scientific Research of the American Medical Association.

average figure was used. Every effort was made to give attention to all essential details, so that experimental conditions were kept as uniform as possible.

RESULTS. The accompanying table shows the results obtained. It will be noted that the average emptying time for the six individuals averaged 2.23 hours. After the subjects had eaten a meal twice the size of the standard meal the average gastric emptying time was increased 16.83 per cent. When a meal had been ingested which was three times the size of the standard meal the average gastric emptying time was increased 38.33 per cent. The gastric emptying time in subject number 4 was not materially affected by the size of the meal.

COMMENT. The results show that while the gastric emptying time is prolonged by a large meal in five of the six subjects, the prolongation is by no means proportional to the size of the meal. It is difficult to give a

TABLE 1

The relation of the size of the meal to the emptying time of the stomach in man

NUMBER	STANDARD MEAL		MEAL TWICE THE SIZE OF NORMAL			MEAL THRICE SIZE OF NORMAL		
	Number of tests	Emptying time	Number of tests	Emptying time	Increase	Number of tests	Emptying time	Increase
		hours		*hours*	*per cent*		*hours*	*per cent*
1	11	2.62	3	3.25	24	2	3.50	34
2	13	1.87	4	2.25	20	2	2.67	43
3	3	1.75	4	2.19	25	2	2.75	57
4	4	2.90	4	3.00	4	2	2.90	0
5	5	1.62	3	2.00	23	2	3.00	83
6	5	2.62	2	2.75	5	2	3.50	13
Average....	6.83	2.23	3.33	2.57	16.83	2	3.05	38.33

satisfactory explanation of the actual causation of this for, as McCann (3) has stated, the real mechanism of stomach evacuation is not well understood. There are many factors which are capable of influencing gastric emptying and there may be rather wide variations in normal individuals as shown by Van Liere and Sleeth (1).

It is quite probable that the distention caused by the large meal tended to increase the effectiveness of the gastric contractions. This could be explained by the fact that the smooth muscle fibers of the stomach were distended and put under a greater tension. It is known that increased tension of a smooth muscle fiber, up to a certain maximum, is capable of producing a more efficient contraction.

The stomach furthermore is probably as capable of extruding, for example, 10 cc. of chyme as it is 5 cc. at each contraction. That is, the vigor of the contraction is the important factor. More and more evidence

points to the fact that under normal conditions the pylorus probably does not greatly influence stomach emptying. Recent work by Crisler and Van Liere (4) corroborates earlier work done on this point.

Apparently subject number 4 has a somewhat different gastric mechanism than the other five subjects for an increase in the size of the meal did not materially affect his gastric emptying time. There was a delay of 4 per cent in the gastric emptying time when the meal twice as large as the standard meal was eaten, but this was within the limits of experimental error for when he ate a meal three times the size of the standard meal his gastric emptying time was found not to be prolonged. In this instance we were apparently dealing with a stomach in which the gastric motility was directly proportional to the size of the meal, that is, the larger the meal the more effective the stomach contractions. It must be stated here that the stomach of subject number 4 was normal in size and contour under the fluoroscope. It would be of interest to know what percentage of individuals have stomachs which show a similar behavior.

It must be emphasized, however, that a prolongation of 38.33 per cent is a pronounced prolongation. The observations made in this paper can explain, in part at least, the reason for delayed hunger contractions after a large meal.

SUMMARY AND CONCLUSIONS

The gastric emptying time was determined fluoroscopically in six young healthy male subjects. The standard meal consisted of 15 grams of Quaker Farina, 1 gram of sodium chloride and 50 grams of barium sulphate. After the normal gastric emptying time had been determined, a meal twice the size of the standard meal was given. It was found that the gastric emptying time was prolonged 16.83 per cent in five of the six subjects. When a meal three times the size of the standard meal was given the gastric emptying time was prolonged 38.33 per cent again in five of the six subjects. One subject was refractory, that is, the size of the meal apparently had no effect on gastric emptying time—at least within the limits of our investigation.

It is suggested that the increased amount of food causes a distention of the stomach which stimulates it to more effective contractions.

This paper lends experimental evidence to the well recognized fact that the onset of hunger contractions in the majority of people does not appear as soon after a large meal as after a small one.

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VARIATIONS IN THE EXTRACTION OF UREA BY THE KIDNEY AND THEIR RELATION TO THE AMOUNT OF UREA REABSORBED[1]

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Received for publication January 9, 1937

The urea content of blood drawn from the renal vein in dogs was found by Van Slyke, Rhoads, Hiller and Alving (1934) to be usually from 6 to 12 per cent less than the simultaneous urea content of arterial blood from the same animal. Such a difference has been designated as the "extraction" by Sheehan (1931). At times the extraction was found to be far outside these limits. Van Slyke et al. commented particularly on those instances in which the urea content of the renal vein blood was equal to or greater than that of arterial blood. They believed that increased reabsorption rather than diminished blood flow was the mechanism, since the oxygen consumption of the kidney remained normal; and that a reflex initiated by trauma incident to puncture of the vein might have precipitated its occurrence. They also considered the possibility of contamination of renal vein blood with urine from accidental puncture of the ureter. Admittedly the phenomenon of suspension of urea extraction was not adequately explained. Furthermore, the unusually high extractions, as well as the low and negative ones, require explanation. The purpose of the present paper is to present evidence concerning the nature of variations in reabsorption and filtration based upon the simultaneous extraction percentages and clearances of creatinine and urea in dogs prepared by a technique which eliminates for practical purposes the possibility of contamination of the blood with urine.

The dogs used by Van Slyke, Rhoads, Hiller and Alving (1934) were prepared according to the technique of Rhoads (1934), who devised the method by which access to the renal vein was permitted at a time when the dog was intact, conscious, and free from pain. Some modifications of Rhoads' technique have since been developed which make it possible to

[1] This work was aided by a grant from the Douglas Smith Foundation. A preliminary report of this work was presented before the meeting of the National Academy of Sciences, November 18, 1936.

avoid the danger of puncture of the ureter or renal pelvis. Sheehan (1936) isolated the renal vein by means of a skin tube, thus interposing a substantial barrier between the renal vein and the other structures of the pedicle. The present authors, aware of his idea, but unfamiliar with the details of his operation, developed a different modification on Sheehan's principle. The operation described here appears to offer some advantages in simplicity and in diminished danger of necrosis of the flaps.

METHODS. The dogs were prepared by explantation of the left kidney and subsequent right nephrectomy. The technique of explantation was as follows:

A skin incision four to five inches in length was made along the tenth intercostal space extending back to the lateral vertebral processes. Large subcutaneous pockets were made both anteriorly and posteriorly (fig. 1A). The kidney was delivered and any defect in the peritoneum was closed with plain catgut. After the vein had been isolated and the kidney fixed in the posterior subcutaneous pocket, the dissection of the flaps was laid out (fig. 1B). A bridge of skin $\frac{3}{4}$ to 1 inch wide was left across the kidney pedicle, and on either side a pyramidal-shaped flap was cut with the broad base away from the bridge. The narrow end, marking the lateral border of the bridge, was about $\frac{1}{2}$ inch wide, and the distal end about $1\frac{1}{2}$ inches wide. The lower (caudal) flap was made about $2\frac{1}{2}$ inches long, while the upper (cephalic) one was about $1\frac{1}{2}$ inches long. The purpose of this difference in length was to avoid compression of the vein by insuring that the sutures did not lie immediately under it when the flaps were joined. The caudal flap was then drawn under the vein and sutured to the cephalic one with silk (fig. 1C). Additional sutures were placed along the edges of the flaps, fixing them to the deep tissues and thus relieving tension on the vein. It was sometimes necessary to incise the muscle and fascial layers underlying the kidney to loosen the vein further. Care was taken to avoid compression of the vein at any stage. The edges of the skin bridge overlying the vein were sutured loosely to the potentially hairy surface of the flaps under the vein. No attempt was made to produce a complete tube of skin around the vein. In spite of the fact that this procedure involved the burying of epithelium, no cysts have been found in dogs autopsied one to three months after operation. The original incision was closed with interrupted silk sutures. The post-operative course in our animals has been uneventful.

The dogs were fed the low protein diet described by Jolliffe and Smith (1931) or a mixed diet including meat. All experiments were carried out after a fast of 18 hours or more. A saline solution containing creatinine, and in some instances urea also, was injected intravenously throughout the experiment with the pump devised by Jacobs (1934). A creatinine solution containing 1.5 to 4.0 grams was administered rapidly and followed by

a solution containing 0.4 to 1.2 per cent creatinine at a rate of about 1 cc. a minute. The concentration of the creatinine solutions and the rate of injection were chosen for each dog to maintain a plasma creatinine concentration of 30 mgm. per cent or more. When the concentrations were properly adjusted, an approximate equilibrium was reached in 30 minutes; experiments were begun after 50 minutes or more. Urine was collected by indwelling catheter and lavage of the bladder for periods of 30 to 60 minutes. Blood was drawn at the midpoint of each period from the femoral artery and from the renal vein as nearly simultaneously as possible,

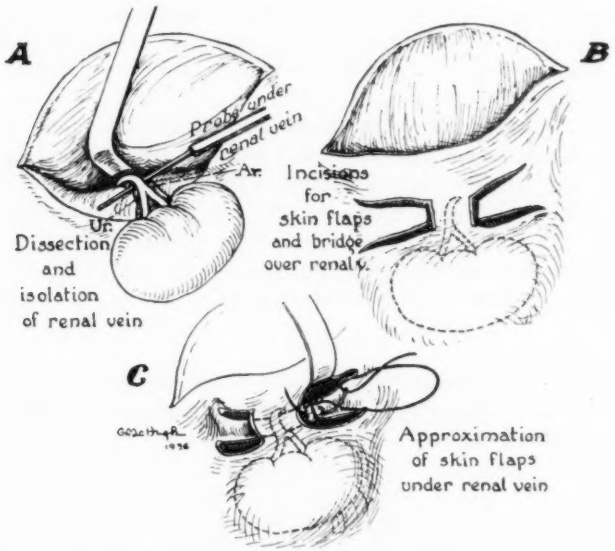

Fig. 1. Operation for explantation of the kidney. *Ar* indicates renal artery; *Ur*, ureter.

usually within a half-minute. A portion of each specimen was centrifugated immediately.

Urea was determined by the method of Van Slyke (1927) in urine and in whole blood. Creatinine was determined in urine, in plasma, and in blood by the method of Folin and Wu (1919). Cell and plasma volume were determined with the Wintrobe (1929) hematocrit. All analyses were done in duplicate, and in triplicate when urea determinations did not agree within 0.1 mgm. per 100 cc. and creatinine determinations within 0.5 per cent.

CALCULATION OF DATA. Present evidence supports strongly the view

that in dogs creatinine is removed from the plasma entirely by filtration through the glomeruli and that none of it is reabsorbed. It may be assumed that a fraction of plasma urea is filtered which is equal to the fraction of plasma creatinine filtered. A portion of the filtered urea and substantially all of the filtered water are reabsorbed by the tubules; and finally urea diffuses from blood cells to plasma in such amounts that the urea is distributed between cells and plasma in proportion to their water content. For data in support of these assumptions, reference is made to Van Slyke, Hiller and Miller (1935), who presented equations which they applied to the approximate mean values for extraction percentages. The means of the present data agree closely with the means of these authors.

Let E_{Ur} = blood extraction of urea, per cent of arterial blood urea
E_{Cr} = plasma extraction of creatinine, per cent of arterial plasma creatinine
V_c, V_p = volume of cells or of plasma in 1 volume of blood
R = urea reabsorbed at the time of taking blood specimens, expressed as a fraction of the amount filtered.
R_M = urea reabsorbed, average for a urine collection period, expressed as a fraction of the amount filtered.

The theoretical plasma urea extraction percentage if cell urea did not diffuse is represented by the following quantity:

$$E_{Cr}\,(1 - R)$$

Because of the diffusibility of urea the observed plasma extraction is equal to the blood extraction. If allowance is made for the diffusion from cells to plasma the equation including the observed extraction percentage becomes:

$$E_{Ur} = \frac{E_{Cr}\,(1 - R)}{1 + 0.75\,\dfrac{V_c}{V_p}}$$

This equation was solved for $E_{Cr}\,(1 - R)$, the theoretical plasma urea extraction, and for R, the urea reabsorption prevailing for the short period of time during which the blood samples were being drawn.

The average reabsorption of urea during a period of 30 to 60 minutes was estimated from the clearance ratio, the reabsorption being represented by the difference between the clearance ratio and unity:

$$R_M = 1 - \frac{\text{Plasma urea clearance}}{\text{Plasma creatinine clearance}}$$

Plasma urea clearance was estimated from the blood clearance and hematocrit readings on the assumption that the cell urea concentration was 0.75 times the plasma urea concentration.

These equations, which formerly have been applied to mean values, are in this work applied to individual values.

RESULTS AND DISCUSSION. A total of 50 experiments was carried out on 6 female dogs. Detailed data for 6 representative experiments, and mean values and standard deviation for all experiments, are given in table 1. Comparative data illustrated by individual experiments are shown in figures 2 and 3.

Creatinine extraction. The plasma creatinine extraction varied from 11.5 to 26.0 per cent (fig. 2). The mean was 18.5 per cent and the standard deviation 3.8. While the limits of variation are fairly wide in the entire series, a given dog on a particular day generally showed little variation.

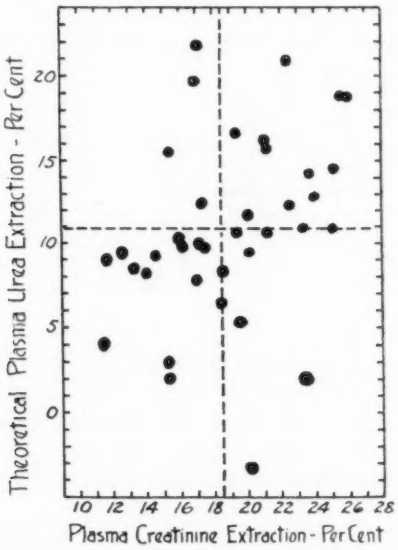

Fig. 2. Theoretical plasma urea extraction and plasma creatinine extraction. Each point represents one experiment; the means are represented by the broken lines.

Urea extraction. Blood urea extraction varied much more than plasma creatinine extraction. The range was from −1.9 to 14.9 per cent, the mean 7.1 per cent, and the standard deviation 3.8. When allowance was made for different amounts of urea diffused from cells to plasma by calculation of the theoretical plasma extraction a similar degree of variation was found. The range of theoretical plasma extraction (fig. 2) was from −3.3 to 21.8 per cent, the mean 10.9 per cent, and the standard deviation 5.4. Neither the observed nor the theoretical urea extraction was substantially more constant on a given day than from day to day.

Urea reabsorption. The values for the reabsorption of urea for the short periods at the time blood specimens were taken varied within wide limits, from −27 to +116 per cent (fig. 3). The mean was 40.9 per cent and the standard deviation 27.4. Reabsorption calculated from the clearance ratios, representing the average for a period of 30 to 60 minutes, had a mean value not greatly different, but varied within much narrower limits (fig. 3). The range was from 24 to 62 per cent, 32 of 36 values lying between 40 and 60 per cent; the average was 47.1 per cent and the standard deviation 7.9. No relation was found between the reabsorption at the midpoint of a period and the average reabsorption for the period.

Variations in extraction percentage as related to other factors. The proportion of the plasma urea which is removed during its passage through the

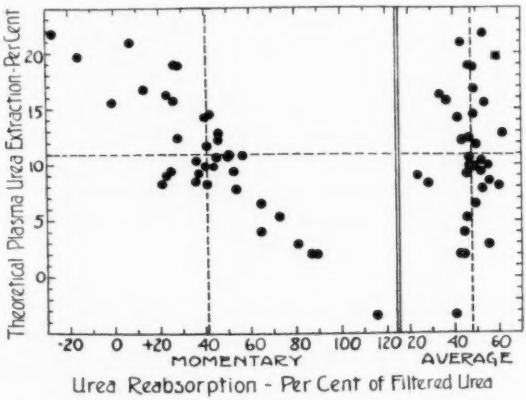

Fig. 3. Theoretical plasma urea extraction and urea reabsorption. Each point represents one experiment; the means are represented by the broken lines.

kidney could possibly be increased or diminished by variation in the proportion filtered or by variation in the amount reabsorbed. Figure 2 shows the relation between the creatinine extraction, which is used as a measure of filtration, and the theoretical plasma extraction of urea. Graphs of creatinine extraction and observed urea extraction are essentially similar but correlate slightly less well, presumably because variation in the cell content influence the observed extraction independently of renal factors. There is a correlation of only 0.34 between the creatinine and the theoretical plasma urea extraction percentages.

Comparison of the momentary reabsorption with the theoretical plasma urea extraction (fig. 3) indicates a high degree of correlation between these quantities, with a coefficient of −0.88. A graph of observed urea extraction and momentary reabsorption is similar. No relation could be found

between the average reabsorption for a period and the urea extraction, either of the blood or of the plasma alone. These observations indicate that, while variations in the percentage of urea extracted from the blood are due in some measure to differences in filtration, the observed variations in extraction are to be ascribed chiefly to differences in the rate at which urea is reabsorbed. Such variations are momentary (fig. 3).

No information is available as to the momentary rates of renal blood flow or of water excretion in these experiments. Neither momentary nor average reabsorption was correlated significantly with the average rate of renal blood flow (estimated from blood creatinine extraction and excretion) or with the average rate of urine excretion for a period.

TABLE 1

Representative experiments

NUMBER OF EXPERIMENT	DOG	DATE	ARTERIAL PLASMA CREATININE	PLASMA CREATININE EXTRACTION	ARTERIAL BLOOD UREA	BLOOD UREA EXTRACTION	MOMENTARY UREA REABSORPTION	THEORETICAL PLASMA UREA EXTRACTION	AVERAGE UREA REABSORPTION	REMARKS*
			mgm./100 cc.	*per cent*	*mgm./100 cc.*	*per cent*	*per cent*	*per cent*	*per cent*	
16	D	7-28	42.0	19.6	8.86	3.7	73	5.3	46	L
17	D	7-28	38.8	17.0	10.12	14.3	−16	19.7	59	L
24	D	11-12	45.8	21.2	14.80	9.1	26	15.7	37	M
39	F	6-23	50.2	14.6	17.64	7.0	37	9.2	46	LA
44	G	11-5	35.9	20.2	5.28	−1.9	116	−3.3	41	L
50	H	11-3	66.3	0.0	14.85	−0.7		−0.9		Anuria L
Summary of all experiments										
Number of experiments			47		49	38	38	36		
Mean			18.53		7.066	40.92	10.89	47.08		
Standard deviation			3.85		3.83	27.36	5.45	7.87		
S.D./Mean			0.208		0.542	0.669	0.500	0.167		

* L = low protein diet
M = mixed diet
A = $CaCl_2$ acidosis

Attention has been called previously to occasions when the urea content of renal vein blood has been equal to or greater than that of the arterial blood. These fall into three groups.

1. Extraction percentages substantially zero could result from a complete suspension of renal activity. One experiment was performed in a dog with anuria (expt. 50, table), in which both creatinine and urea extraction percentages were zero. A complete cessation of glomerular filtration occurred in this instance.

2. Apparent addition of urea to the blood during its passage through the kidney occurred twice, and removal was less than 2 per cent three times.

In none of these experiments could the mechanism have been a suspension of filtration, for creatinine extraction was of the usual magnitude. The estimated momentary urea reabsorption in these experiments was therefore in the neighborhood of 100 per cent. The negative extraction of Addis and Shevky (1917), and three of the four negative extractions of Van Slyke, Rhoads, Hiller and Alving (1934) were, like ours, less than 2 per cent. Complete reabsorption of urea could account for extractions of zero. Moreover, plasma water is not always completely reabsorbed, since a fraction equal to the urine volume must perforce be excreted. In this group of experiments and in those cited the magnitude of the negative extraction has not been outside conceivable limits of hemoconcentration.[2] A slight negative urea extraction percentage may be apparent rather than real, but there can be no doubt that substantially complete reabsorption of urea does occur for short periods.

3. Extractions from -1 to -5 per cent have been found in the anesthetized rabbit by Kay and Sheehan (1933). All were observed during a period from 16 to 26 minutes after the injection of a large dose of urea, and followed a period of exceptionally great extraction. They are probably characteristic of a phase in the establishment of equilibrium between the urea content of the renal cells and that of the blood, rather than of any condition occurring physiologically. Positive extractions comparable to those in the present report were found after equilibrium was established. The single negative extraction of 27 per cent reported in an unanesthetized dog by Van Slyke, Rhoads, Hiller and Alving (1934) can be explained as an artefact. If such an extraction be accounted for on the basis of reabsorption the values for creatinine extraction and plasma volume in this series would demand urea reabsorption of 220 to 380 per cent of the amount filtered. In view of the rarity of the finding and the degree of deviation from all other observations, it is likely that contamination of the renal vein blood with urine is the correct explanation.

Experiments were carried out under conditions which might logically be conducive to disturbances in reabsorption of urea. The renal vein was occluded completely by digital pressure continuously or intermittently for periods as long as 10 minutes and the extraction was estimated during and after that procedure. On one such occasion a urea extraction percentage of urea of zero was found after release of the pressure; at all other times extraction was of the usual magnitude.

[2] Van Slyke et al. (1934) compared hemoglobin concentrations in the arterial and renal venous blood. Though such comparisons are admittedly of value in detecting changes in blood concentration produced by fluid exchange within the kidney, conclusions based on them can not be accepted entirely without reservation because the hemoglobin concentration of the renal venous blood can conceivably be influenced by transient storage or discharge of red blood cells due to vascular changes within the kidney.

Unusually high extraction of urea from the blood, associated with unusually low urea reabsorption, was encountered as frequently as unusually low extraction, and was equally irregular and unpredictable in its occurrence. On three occasions reabsorption was negative; that is, more urea apparently disappeared from the blood stream than could be accounted for by the amount presumably filtered. At least one of these results was within the ordinary limits of error in the method. The other two would seem to signify active secretion of urea; however, we believe the number of observations too few and the possible errors too great to justify such a conclusion.

Physiologic significance. With the exception of two experiments in which negative reabsorption was found, and which remain unexplained, these results are in accord with the filtration-reabsorption theory of urine formation. They suggest that fluctuation from complete reabsorption of urea to no reabsorption at all occurs physiologically, and that by chance blood may be drawn at a time when reabsorption is either greater or less than its mean value. That the momentary reabsorption found in the present experiments portrays physiological conditions is supported by the fact that the mean momentary reabsorption (41 per cent) is near the mean of the reabsorption over long periods (47 per cent). The difference is probably not significant; if it is significant at all it tends to the view that puncture of the renal vein disposes toward diminished rather than increased reabsorption, or toward increased rather than diminished urea extraction percentage. The theory that temporary diminution or disappearance of urea extraction is due to a reflex from the puncture of the renal vein fails to account for fluctuations in the opposite direction, which are at least equally great and equally frequent. Hence, if variations in reabsorption are due to reflex activity, such activity must exist independently of the experimental manipulation, unless it be assumed that two opposite reflexes may occur from what is apparently a single form of stimulus. In spite of wide momentary fluctuations in the urea reabsorption the average reabsorption over a 30 to 60 minute period is relatively constant; hence the transitory variations, though of physiological interest, are of slight or no importance in determining the rate of urea excretion over a period of time.

These results explain the observation of Van Slyke (1935) that renal blood flows calculated from creatinine extraction are more satisfactory than those calculated from urea extraction.

CONCLUSIONS

The following conclusions are based upon data from 50 experiments in one-kidney dogs in which the kidney is explanted by a modified Rhoads technique to insure collection of uncontaminated blood from the renal vein.

1. Momentary variations in the extraction percentage of urea are

dependent chiefly upon changes in the rate at which urea is reabsorbed, and to a relatively unimportant degree upon changes in the rate of filtration.

2. The rate of reabsorption varies from none to all of the filtered urea and the average lies between 40 and 50 per cent; this average is usually approximated within any period of 30 to 60 minutes.

3. Cessation of the extraction of urea due to cessation of filtration can occur in association with anuria.

4. Increase in urea concentration of the blood in the kidney does not exceed that explicable by complete reabsorption of filtered urea.

5. It is postulated that extreme fluctuations in the rate of reabsorption of urea occur independently of experimental procedures.

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THE NERVOUS SECRETION OF SALIVA: QUANTITATIVE STUDIES IN THE NATURAL UNCONDITIONED REFLEX SECRETION OF PAROTID SALIVA[1]

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Received for publication January 12, 1937

The work of Pavlov has shown us that the amount of secretion from the stomach and pancreas is directly proportional to the weight of food ingested. Thus with 100 grams of meat there is twice as much gastric and pancreatic juice as with 50 grams (1). But in spite of the many thousands of experiments in Pavlov's laboratory involving the parotid and submaxillary secretion, there have been none to show how these glands react to the amount of food. The explanation probably lies in the novelty and fascination of the work with the conditioned reflexes, as well as to the lack of physiological importance of the salivary glands compared with the stomach and pancreas.[2]

However, the salivary glands present several advantages for the study of the nervous control of secretion. First, there is no complication from a hormonal or chemical secretion as there is in the stomach from contact of food with the mucosa. Secondly, collection and measurement of the saliva is more accurate, making possible the use of very small quantities of food, and introduction of factors that may lead to an analysis of secretion more readily than is the case with other glands. In addition the salivary gland has been made the basis of the animal work on the conditioned reflex, upon which has been built a quantitative psychology. A corresponding *quantitative physiology* is now essential for an understanding of the laws of the conditioned reflexes as well as for the sake of normal physiology of secretion. Furthermore the same methods and preparation (the normal animal) should be used in the study of both conditioned and unconditioned reflexes to obtain comparable results.

The results of this study are based upon more than 8,000 recorded

[1] Read at the meeting of the American Physiological Society, Washington, D. C., March, 1936.

[2] Babkin showed that the volume of parotid saliva to acid depends upon the concentration of the acid (Thesis, St. Petersburg, 1904). Kupalov and Gantt proved that the quantity of saliva and other digestive juices depends on the state of division of the food, as well as on the composition (2).

TABLE 1

DOG, SEX, YEAR OF BIRTH, WEIGHT IN KGM.	PERIOD OF EXPERIMENTS	NUMBER OF OBSERVATIONS TO EACH AMOUNT FED	PRECEDED BY C.R. (+), NOT PRECEDED BY C.R. (−)	AMOUNT AND KIND OF FOOD (SPRATTS) AND (PURINA)	AMOUNT OF SECRETION	SECRETION PER GRAM OF FOOD PER KGM. BODY WEIGHT	LATENT PERIOD	a	$k = \sqrt{Q8}$/gm./kgm.
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
				grams	*cc.*	*cc.*	*seconds*	*cc.*	
				Spratts					
Pat	April, 1934	15	−	2.0	0.51	0.013	3.1		0.018
m.				4.0	0.59	*0.008*	2.9	0.41	0.016
1931				8.0	0.79	0.005	2.7		0.014
19 kgm.				16.0	1.23	0.004	2.7		0.016
				Spratts					
Maxine	Dec., 1934	20	−	0.5	0.31	0.024	2.4		0.017
f.				1.0	0.37	0.015	2.1		0.015
1932				2.0	0.41	0.008		0.27	0.011
20 kgm.			+	4.0	*0.68*	*0.007*			0.014
				8.0	0.98	0.005	1.8	0.33	0.014
	May, July, 1935	80	+	2.0	0.41	0.008			0.011
				12.0	0.82	0.003			0.010
				Spratts					
Riji	Jan., Feb., 1936	32 to 48	−	2.0	0.39	0.008	5.1		0.010
m.				4.0	*0.60*	*0.006*	4.7		0.012
1929?				8.0	0.81	0.004	4.9	0.25	0.011
25 kgm.				16.0	1.34	0.003	4.9		0.012
			−	16.0	0.97	0.0024	4.0		0.010
				24.0	1.30	0.0022	3.8	0.17	0.011
				32.0	1.76	0.0022	4.4		0.012
				Spratts					
Polkan	1933–34	20	−	2.0	0.66	0.0165	4.8		0.023
m.				4.0	*0.69*	*0.008*	5.7	0.53	0.016
1930				8.0	1.33	0.008	4.8		0.022
20 kgm.				16.0	1.59	0.005	4.3		0.020
				Spratts					
No. 3	1932–34	700	+	2.0	0.11	0.005		0.06	0.007
f.				12.0	0.38	0.003			0.010
1 yr.									
8 kgm.	Feb., 1932		−	8.0	0.12	0.0015	8.2		0.004
				Purina					
			−	12.0	0.09	0.0007	7.5		0.002

TABLE 1—*Concluded*

DOG, SEX, YEAR OF BIRTH, WEIGHT IN KGM.	PERIOD OF EXPERIMENTS	NUMBER OF OBSERVATIONS TO EACH AMOUNT FED	PRECEDED BY C.R. (+), NOT PRECEDED BY C.R. (−)	AMOUNT AND KIND OF FOOD (SPRATTS) AND (PURINA)	AMOUNT OF SECRETION	SECRETION PER GRAM OF FOOD PER KGM. BODY WEIGHT	LATENT PERIOD	a	$k = \sqrt{QS}$/gm./kgm.
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
				grams	*cc.*	*cc.*	*seconds*	*cc.*	
				Purina					
Billy m. 1928? 20 kgm.	1931	30	+	2.0	0.36	0.009		0.29	0.013
				24.0	1.15	0.0024			0.012
	1932	1360	+	3.0	*0.50*	0.008	4.3	0.37	0.014
				18.0	1.13	0.003	2.5		0.013
	1933	455 ea.	+	1.5	0.51	0.017		0.43	0.021
				18.0	1.44	0.004			0.017
	Apr., May. 1935	280	+	0.75	0.41	0.027		0.37	0.023
				18.0	1.33	0.004			0.017
	Feb., March, 1936	60	+	6.0	0.84	0.007	2.9		0.017
				12.0	1.26	0.005	3.0		0.017
				24.0	2.12	0.005	3.0	0.44	0.024
				36.0	2.85	0.004	3.1		0.024

measurements in 12 dogs, continuing, in some of the animals, over a period of 5 years. The material is presented to fill a gap in the physiology of parotid secretion as well as to furnish a basis for a further analysis of the relationship between the conditioned and the unconditioned reflexes.

METHOD. The method used was that of the chronic fistula, introduced by Pavlov for the study of the various digestive juices. Such a technic gives us the possibility of examining the intact normal animal over a long period—the advantage of a physiological instead of a pathological preparation. Twelve dogs were employed, all of which had a parotid fistula of the left gland. The animals were tested according to the routine of the conditioned reflex experiment; they were enclosed in the *camera silenta* and fed every 2 to 5 minutes automatically by dropping the food into the box in front of the animal. The unconditioned reflex was measured for 60 seconds from the time the food was placed before the dog; the secretion is practically always finished by the end of 20 to 45 seconds depending on the amount of food and on the individual animal.

Two kinds of food were used: (only one kind in each animal, except for special comparison) "Spratts ovals" and "Purina checkers." The former gives more than twice as much secretion as the latter (see dog 3 in table 1).

In general Spratts is an excellent food for conditioned salivary reflex experiments owing to the large amount of secretion provoked, the uniform size of the biscuits, and the readiness with which the dog eats it.

In some of the dogs (*Blue, Fips, Lady, no. 3*) the feeding was preceded by a conditioned signal for 10 seconds; in *Riji, Maxine, Pat, Polkan, Fritz, Ephraim,* there was no preceding conditioned reflex; in *Billy* and *Kompa* a combination of the two methods was applied. These measurements do not include the conditioned reflex except that part of it which might continue after eating begins, as discussed later.

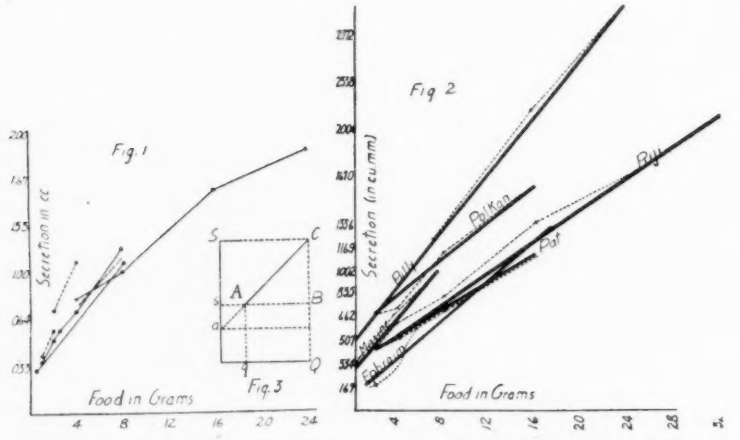

Fig. 1. Variation of unconditioned reflex secretion at different periods of dog's life.

Fig. 2. Showing relation between size of unconditioned reflex and amount of food.

Fig. 3. Derivation of formula for quantity of food and secretion.

The amount of food varied from 0.5 gram to 40 grams at each feeding. We began these experiments by giving a quantity of food at each experimental feeding for a period of 12 to 24 months and gradually reducing it for another similar period (Kompa) in order to rule out fluctuations. This process, however, proved not only slow and laborious, but inaccurate because of the changing age, and physical state of the animal, so that it happened with one dog (Kompa) that there was no more secretion to 4 grams of food than to 8 grams given at an earlier period. See figure 1. The most accurate method is to give all the quantities with which comparisons are made on the same day in a regular periodic sequence. The following protocol illustrates this method:

Billy, March 9, 1936

TIME	LATENT PERIOD SECRETORY	AMOUNT OF FOOD	SIZE, UNCONDITIONED REFLEX
	seconds	*grams*	*cc.*
13.50	5.2	6	1.06
13.52	5.0	12	1.24
13.54	3.8	24	1.86
13.56	4.2	36	3.16
13.58	4.0	6	0.89
14.00	3.2	12	1.11
14.02	4.4	24	1.52
14.04	4.0	36	3.09
14.06	4.2	6	0.86
14.08	4.0	12	1.28
14.10	4.2	24	1.79
14.12	3.8	36	3.01

Av. 6 grams = 0.93 cc. Av. 24 grams = 1.73 cc.
Av. 12 grams = 1.21 cc. Av. 36 grams = 3.09 cc.

Only two quantities of food were used in no. 3, Lady, Fips. In Billy for several years only two quantities were given, later one only of the quantities was reduced and the new pair of feedings given together for another year or more, so that we had a combination of (1) 18 grams with 2 grams, (2) 18 grams with 1.5 grams, (3) 18 grams with 0.75 gram. Finally Billy was given a series of four quantities, as shown in the above protocol.

The weight of each biscuit averaged 4 grams for the Spratts and 6 grams for the Purina checkers. An amount equal or less than the weight of a single biscuit was given undivided, as one piece, but an amount greater than the weight of a biscuit was given in more than one morsel; *e.g.*, 1, 2 or 4 grams (Spratts) means a single piece, but 8, 16, or 20 grams is two, four or five pieces respectively.

RESULTS. Plotting the results with the quantity of food in grams as abscissae and the parotid secretion in cubic centimeters as ordinates, it is seen that the graphs are all straight lines. They cut the perpendicular axis at a point representing 0.13 cc. in Lady ranging up to 0.50 cc. in Polkan; the average of all the dogs is about 0.40 cc. (see fig. 2). The height of this point (a) represents then the (conditioned?) secretion to a minimum amount of food. For any larger amount of food the quantity of secretion can be calculated by multiplying the weight of food in grams by a fraction b. Both a and b are quantities characteristic for each individual animal. b varies from 0.01 in Lady to 0.09 in Kompa and Pat, for most of the dogs $\frac{ds}{dq} = 0.05$ as shown in table 1. In other words, the secretion varies only $\frac{1}{20}$ as fast as the amount of food. There is no essential difference in the computation for the two kinds of food used.

The amount of parotid secretion is expressed by the formula $S = a + bQ$ where S = parotid secretion in cubic centimeters (from 1 gland), a and b = constants characteristic of each dog, and Q = weight of food in grams. The slant of the curve, i.e., rate of increase, in each dog can be calculated from the relation between the secretion for the large and small amounts of food by the formula:

$$b = \frac{S - s}{Q - q}$$

where S = secretion to large amount food in cubic centimeters.
s = secretion to small amount food in cubic centimeters.
Q = large amount food in grams.
q = small amount food in grams.

The derivation is as follows:
Let s and S be the points on the line aAC representing the secretion with q and Q grams of food respectively (fig. 3).

then

$$S - s = CB$$

and

$$Q - q = AB$$

but

$$\tan \cdot \angle CAB = \frac{CB}{AB} = \frac{S - s}{Q - q}$$

If the secretion is reduced to cubic centimeters per gram of food and the values are plotted with the corresponding quantities of food (column 5, table 1) we get a series of hyperbolas (fig. 4). The equation for these hyperbolas is

$$S' = \frac{a}{Q} + b \qquad (1)$$

where S' is secretion per gram of food, a and b are constants. If we further reduce the secretion to cubic centimeters per gram of food per kilogram body weight we find that the hyperbolas nearly coincide, as shown in figure 4; this means that the constants a and b are proportional to the weight of the dog.

I am indebted to Prof. N. Rashevsky, biomathematician of the University of Chicago, for the following analysis:

"The coincidence of the hyperbolas indicates that the quantities a and b of the equation

$$S = a + bQ$$

are proportional to M, the total weight of the dog. Consider the secretion per gram of food per kilogram body weight:

$$S_1 = \frac{S'}{M} \tag{2}$$

Combining (1) and (2) we have

$$S_1 = \frac{a}{MQ} + \frac{b}{M} \tag{3}$$

If

$$a = k_1 M \quad \text{and} \quad b = k_2 M$$

or

$$\frac{a}{M} = k_1 \quad \text{and} \quad \frac{b}{M} = k_2$$

where k_1 and k_2 are constants independent of the dog, then from (3) we have

$$S_1 = \frac{k_1}{Q} + k_2$$

for any dog, as actually found in the experimental data." Moreover it is fairly constant at a given period in any dog within limits of 1 to 36 grams of food, *e.g.*, see Riji, Pat, Maxine. At different periods of the dog's life, there may be considerable variation, *e.g.*, Blue in 1931 (0.011) and 1931–32 (0.021, 0.035). See figure 1.

The secretory latent period varies from about 2 seconds in Maxine to about 5 seconds in *Riji* and *Polkan*. In table 1 is given the latent period corresponding to each amount of food. It can be seen that there is no variation of latent period with the amount of food.

Our latent period does not represent the exact latent period of the parotid gland, as some time is necessary for an accumulation of saliva sufficient to overcome inertia and friction of the system, but the absence of variation from the latent period corresponding to the smallest amount of food to that of the largest would indicate that this element is small. The fact that our system is completely waterfilled, and therefore relatively incompressible compared to an air system is additional evidence that our

latent period probably approximates that in the duct of the gland (unless microscopic quantities are considered). It is only $\frac{1}{6}$ of the external latent period of the nervous secretion of gastric juice in the dog (3 to 5 min.).

The actual latent period for the salivary conditioned reflex has been shown by Volinsky to be 0.04 to 0.08 second to a bell whose external latent period was 1 to 1.5 seconds, and the latent periods to weak conditioned stimuli such as a dim light were from 0.1 to 0.2 second, while the external latent period was from 3 to 5 seconds.

Table 1 summarizes the results in the ten dogs.

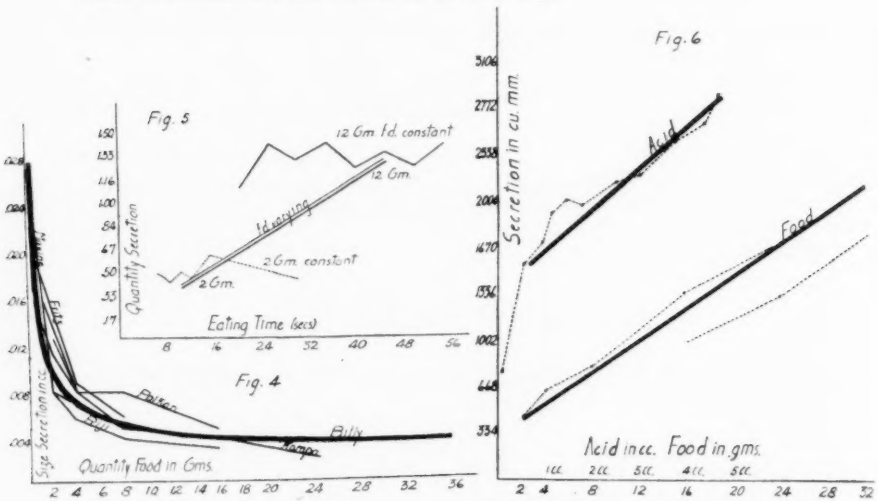

Fig. 4. Secretion per gram of food per kilogram body weight for varying quantities of food.

Fig. 5. Relation between secretion and duration of eating.

Fig. 6. Comparison of curves of secretion to acid and secretion to food.

RATE OF EATING. In most of the animals the time required to eat the food was measured by observing through the window of the camera the moment when the chewing movements ceased. Licking of the food box which often followed deglutition was noted separately but not included with the time of eating. Obviously the estimation of short periods, such as those for small amounts of food contain a greater factor of error than periods for larger amounts. This may account for the irregularity of the curves for eating time as compared with those for quantity of food. A correlation of the "eating time" and the secretion is given in table 2. Part 1 records the time when the amount of food varied; part 2 when the amount was kept constant.

A similar formula to that governing the relation between food and se-

cretion holds for duration of eating (mastication and deglutition) and secretion, when the amount of food varies, *viz.*,

$$S = a_1 + b_1 t$$

where S = secretion,
a_1 = a constant (the minimum secretion)
and b_1 = a constant $\left(\frac{ds}{dt}\right)$.

TABLE 2

Eating time

Part 1 (Food varying)

DOG, SEX, DATE OF BIRTH, WEIGHT IN KGM.	PERIOD OF EXPERIMENTS	NUMBER OF OBSERVATIONS	AMOUNT AND KIND OF FOOD	AMOUNT OF SECRETION	AVERAGE EATING TIME	RATE OF EATING
(1)	(2)	(3)	(4)	(5)	(6)	(7)
			grams	*cc.*	*seconds*	*grams/seconds*
			Spratts			
Pat	April 18–24,	15 each	2.0	0.50	9	0.22
m.	1934	amount	4.0	0.54	10	(*0.40*)
1931			8.0	0.85	18	0.45
19 kgm.			16.0	1.26	21	0.76
			Spratts			
Maxine	Dec., 1934	7 each	0.5	0.31	3	0.17
f.		amount	1.0	0.37	4	0.25
1932			2.0	0.40	4	0.50
			4.0	0.68	11	(*0.36*)
			8.0	0.99	13	0.62
			Spratts			
Riji	Jan., 1936	5 each	2.0	0.60	6	0.37
m.		amount	4.0	0.69	11	(*0.36*)
1929?			16.0	1.49	42	0.38
25 kgm.						
			Spratts			
Polkan	1933–1934	40 each	2.0	0.66	6	0.37
m.		amount	4.0	0.69	11	(*0.36*)
1930			8.0	1.12	17	0.47
20 kgm.			16.0	1.59	25	0.64
			Purina			
Billy		400 each	0.75	0.36	5	0.15
m.		amount	1.5	0.49	6	0.25
1928?			3.0	0.71	8	(*0.37*)
20 kgm.			18.0	1.44	21	0.86

TABLE 2—*Concluded*

Part 2 (Food constant)

DOG, SEX, DATE OF BIRTH, WEIGHT IN KGM.	PERIOD OF EXPERIMENT	NUMBER OF OBSERVATIONS	AMOUNT AND KIND OF FOOD	AMOUNT OF SECRETION	AVERAGE EATING TIME	RATE OF EATING (AVERAGE FOR EACH AMOUNT FED)
(1)	(2)	(3)	(4)	(5)	(6)	(7)
			grams	*cc.*	*seconds*	*grams/seconds*
			Spratts			
Pat m. 1931 19 kgm.	March 4–13, 1934	12 each amount of time	8.0	0.58 0.70 0.85 0.91	3 6 9 13	1.07
Maxine f. 1932			0.5	0.23 0.27 0.22 0.23	2 3 5 6	0.12
			8.0	0.71 0.66 0.70 1.38	7 8 10 14	0.80
			Purina			
Billy m. 1928? 20 kgm.		400	0.75	0.35 0.36	4 6	0.15
			1.5	0.43 0.59	5 9	0.21
			3.0	0.58 0.69 0.84	5 9 13	0.33
			18.0	1.45 1.49 1.50	13 18 23	1.00

That is the time required for the dog to eat the food is proportional to the amount of food:

$$\frac{dq}{dt} = \frac{b_1}{b}$$

From table 2 we see that when S and a are in cubic centimeters and t in seconds, a averages about 0.20 cc. and $\frac{ds}{dt} = 0.016$ to 0.067 depending on

the dog. In other words, more time is required to eat a large amount of food, but the secretion in cubic centimeters varies only $\frac{1}{6}$ to $\frac{1}{15}$ as much as the time in seconds.

But if we compare the varying time which the dog takes to eat one amount of food, *i.e.*, where the food is kept constant, we see that there is nearly as much secretion in a given dog when the animal eats the food quickly as when he eats it slowly, even though the period may be three or four times as great in one instance as in the other. Figure 5 shows this.

Column 7, table 2, shows that the rate of secretion is much greater for large than for small amounts of food in most of the dogs. For a given amount of food this rate is remarkably constant in all the dogs but Blue and Lady, *e.g.*, 0.36 to 0.44 gram per second for a 4 gram morsel. In Blue it was only one-half this rate and in Lady over twice this rate.

Discussion. In the human one may not conclude that the secretion of saliva is necessarily independent of the time spent in eating. First, it appears from our experiments that although there is no appreciable increase of secretion to the prolonged chewing of a given amount of food, the taking of small mouthfuls would augment considerably the flow of saliva owing to the presence of the factor *a*. Hasty eating is frequently followed by indigestion, probably not because the increased chewing produces more secretion, but because of a different mental state in the two cases. Anxiety and a lack of interest in the food, or preoccupation and certain emotions are known experimentally to inhibit gastric secretion, and most of us have experienced in ourselves the dry mouth of emotional states.

The amount of food varied from 0.25 to 40 grams. At neither end of the curves was there a tendency for the degree of slope to change. That is, between these limits, representing on the one hand, a morsel no bigger than a small pill, and at the other extreme more than could be taken in one gulp or mouthful, the same constant factors are involved. The quantity of food was not further decreased because extreme reduction would require special means of feeding the dog, thereby altering the *milieu* of the comparisons; moreover a point would soon be reached where our chief stimulus would be conditioned instead of unconditioned.

Although it is known that the state of division of the food has an influence on secretion—silicon dioxid in the form of sand, *e.g.*, producing more salivary secretion than in the form of quartz pebbles (4), because it must be ejected, and meat powder more saliva than meat because it must be lubricated for swallowing but requiring less gastric and pancreatic fluids for digestion (2)—it is unlikely that the size of the morsels was sufficiently different in our small and large feedings to be a factor.

As "unconditioned reflex" we have considered in this paper the total flow of saliva ensuing on the eating of the food, actually from the time the

food is taken by the dog. It is possible that there are present some conditioned reflex elements consequent to the sight, smell, sound of the food dropping into the box at the moment of eating. For several reasons (to be discussed more fully in a paper dealing with the effect of the conditioned reflex on the unconditioned) we believe these factors are very small, or negligible at least with the larger amounts of food; for it seems that the unconditioned reflex inhibits the conditioned reflex as soon as the former gets under way. Where there is no specially introduced conditioned signal, but only the unavoidable ones accompanying the presentation of the food, as sight and smell, the time of action of these is very short, as the dog, not having been fed for 24 hours previously, snatches the food avidly and almost immediately.

The question of the various factors that play a rôle after the dog begins eating, *i.e.*, the reflexes arising from the action of eating, is more difficult to analyze. No attempt here is made to evaluate them. For the sake of simplicity we consider the total flow of saliva consequent to the act of eating and independent of the above mentioned preceding conditioned reflexes as "unconditioned." It seems unlikely that there is both a nervous and a chemial secretion for the mouth. The speed of the salivary secretion, its abrupt rise and fall depending on the presence of food in the mouth (excluding the conditioned reflex), would argue against a chemical or hormonal secretion such as exists for the stomach, pancreas and intestinal secretions. But whether there is a "conditioned reflex" (cortical, acquired during the life of the individual) arising from the act of eating, mastication, or the taste or tactile stimulation of the food in the mouth, is too complicated to be solved here. Repeated observations in our laboratory on several dogs show that such factors as the movements of mastication, or for ejecting acid, licking the food box *after* the food has been eaten, produce very little if any secretion when they *follow* the unconditioned stimulus. Thus with Maxine on the 6th of December, 1934 we obtained the secretion by five second periods tabulated on page 505.

It is seen that most of the secretion (72 to 78 per cent) occurs during the period of actual eating (mastication and deglutition), and very little afterwards, furthermore that the *movements* and other accompaniments of licking do not produce any secretion.

The nearly complete absence of secretion during licking (after eating) is evidence that the chief factor in what we have recorded as the "unconditioned reflex" secretion of saliva is the presence of food in the mouth. The existence of the "water reflex"[3] (the flow of saliva to water, as a condi-

[3] That the movements of eating, spewing, licking do not give the secretion is a point against the behavioristic doctrine that the empty peripheral muscular movements are of prime importance; it is essential that the central excitation be present. See Gantt, Katzenelbogen, and Loucks, (3), Light and Gantt (5).

0.5 GRAM FOOD			8.0 GRAMS FOOD		
Time	Amount secretion	Per cent	Time	Amount secretion	Per cent
	cc.			cc.	
0 to 5	0.180 } eating	72 in 10″	0 to 5	0.335 } eating	78 in 10″
5 to 10	0.050 }		5 to 10	0.084 }	
10 to 15	0.015 } licking	9 in 15″	10 to 15	0.016 } licking	2 in 10″
15 to 20	0.015 } food		15 to 20	0.000 } food	
20 to 25	0.000 } pan			pan	
25 to 30	0.015	19 in 35″	20 to 25	0.025	20 in 40″
30 to 35	0.030		25 to 30	0.025	
35 to 40	0.000		30 to 35	0.025	
40 to 45	0.015		35 to 40	0.008	
45 to 60	0.000		40 to 45	0.008	
			45 to 50	0.008	
			50 to 60	0.000	
Total for 60....	0.320			0.534	

tioned stimulus in a dog after acid has been for some time introduced into its mouth as an unconditioned stimulus (3) would indicate that the conditioned response can arise from the mouth as well as from the distant receptors (eye, ear).

In view of the two above mentioned factors (taste and tactile stimulation) which may really function as conditioned stimuli and enter into the "unconditioned reflex" secretion of saliva, it is probably better to use here the term *buccal reflex to food* in preference to the unanalyzed expression "unconditioned food reflex." A step toward this analysis is the work of Finch in this laboratory, excluding from the stomach the food after it has been masticated by letting it escape through an esophageal fistula.

The existence of the quantity *a* in all of the dogs, *i.e.*, the cutting of the ordinate at an appreciable distance above the zero point, in other words the fact of an initial considerable secretion with a minimal amount of food may be explained in several ways. 1. The presence of a certain quantity of saliva in the duct may be squeezed out by the act of mastication. A comparison with the lack of saliva caused by such movements as licking of the food box (see above) indicates that *a* is larger than could be accounted for by this movement. 2. The relatively larger surface of the small amounts of food might be responsible for a greater secretion with small amounts of food, but the uniform straightness of the graphs tends to rule out surface area as a prime factor. 3. The possibility of *a* representing a conditioned reflex has already been discussed. Although it may have a slight influence for the first few seconds, it is, if present, quickly inhibited. The fact that acid introduced into the mouth automatically (without

signals or forewarnings and therefore without preceding conditioned elements) gives an even higher value of *a*, argues against this explanation (see fig. 6). We were not using a quantity of acid that would evoke a maximum secretion as Babkin did in his experiments. This does not, however, rule out any conditioned reflex factor arising from the presence of the acid within the mouth. Also in one of our dogs, Billy, when we reduced the amount of food to 0.75 gram, the conditioned reflex to the signal used for this amount, formerly used as a signal for 3 grams, gradually disappeared so that we arrived at a quantity of food so small that it was unable to evoke an ordinary conditioned reflex from the distant receptors, although it still gave a comparatively large unconditioned reflex. 4. There is further the possibility that we are dealing here with the question of threshold and with an all or none law, such as shown by the work of Adrian, holds for single nerve fibers and muscle contraction, in the sense that the secretion depends upon the number of receptor endings stimulated after the threshold has been attained.

CONSTITUTION. The amount of internal and external secretions has frequently been made the basis for a classification into constitutional types.

The detailed data are given in the tables to bring out individual differences in the dogs. Column 7 and 8 in table 1 give the amount of secretion per kilogram of weight and the latent period for each dog. For purposes of comparison the italicized type in column 7 gives the secretion in cubic centimeters corresponding to 4 grams of food. The range is from about 0.004 to 0.008. There is very little difference in several of our dogs of varying constitution in the secretion to 4 grams food (coefficient of variation = 12), and also little difference in their secretion for 8 and 16 grams (coefficient of variation = 21 and 17 resp.). Figure 4 shows how closely the secretions in all the dogs coincide when reduced to a unit denominator (per kgm. body weight). The secretion would seem to be determined chiefly by the weight of the dog; possibly by the method of eating, as in Lady. No. 3, the other exception, was a pathological animal, having had one-half of the cortex removed.

The rate of eating, as shown in column 7, table 2, is nearly the same for all animals with 4 grams and smaller amounts of food, with the exception of Lady who had a very rapid eating time, and Blue with a slow eating rate. There is considerably more deviation, however, for this rate with larger amounts of food. The coefficient of variation for 4 grams = 27 and for 8 grams = 44. The dogs differed markedly in their external behavior and physical type. Lady was a very energetic dog who bolted her food without chewing, showing little variation in the duration of deglutition and mastication for different amounts of food. Blue was a timid collie who chewed slowly and mincingly. But as the rate of secretion is nearly the same in the other dogs, *viz.*, 0.008 cc. per gram of food per kilogram body

weight, with 4 grams food, no definite correlation can be made between type and amount of secretion.

SUMMARY

1. The secretion of parotid saliva can be expressed as a straight line function of the amount of food (Q) by the equation

$$S = a + bQ$$

where S = secretion in cubic centimeter, a and b = constants, proportional to the weight of the dog. 2. The secretion of saliva with a *given amount* of food per kilogram body weight was remarkably constant in all our dogs but one. It is a hyperbolic function whose equation is

$$S' = \frac{a}{Q} + b$$

where S' = secretion per gram of food, Q = amount of food, a and b are constants. 3. The rate of eating has only a slight effect on the amount of secretion. It is considerably faster for large than for small quantities of food. For a given amount of food (4 grams) it is nearly the same in eleven out of twelve dogs. 4. An analysis of the various factors involved in the secretion of saliva is given, and the data referred to the constitution of the animals.

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THE INFLUENCE OF PSEUDOPREGNANCY ON FOLLICULAR SENSITIVITY TO PREGNANCY URINE EXTRACTS

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Received for publication January 12, 1937

It has long been known that coitus in the pseudopregnant and pregnant rabbit does not induce ovulation. Hammond and Marshall (1925) offer the hypothesis that the corpus luteum and follicle are dependent on the same substance for their development and that the active corpus luteum has a prior call on this substance which prevents complete maturation of the follicle, rendering the ovary incapable of responding to the stimulus of coitus during pregnancy or pseudopregnancy.

This study was designed to investigate the follicular sensitivity of the ovary to pregnancy urine extracts during pseudopregnancy.

METHOD. Pseudopregnancy was induced by coitus with a vasectomized buck or by the injection of pregnancy urine extracts into post partum rabbits. Five, ten or fifteen days after ovulation, checked in every instance by an exploratory laparotomy, one, two or three minimal ovulating doses of pregnancy urine extract were injected intravenously. The rabbits were laparotomized the next day and examined for fresh corpora lutea, corpora hemorrhagica and cystic follicles, but only the presence of fresh corpora lutea was considered a positive reaction. At that time the number of older corpora lutea present as a result of the previous sterile coitus or injection was counted.

The pregnancy urine extracts were obtained through the courtesy of Dr. Oliver Kamm, of the Parke Davis Co., as Antuitrin S. These were kept stored at 4°C. On dilution with distilled water, using a tuberculin syringe and volumetric flask, the extract was assayed according to the method described by Friedman (1932), using post-partum rabbits to determine the minimal ovulating dose in terms of cubic centimeters of pregnancy urine extract per kilogram body weight of rabbit. It has been the finding in this laboratory that post-partum rabbits in good health are uniformly in heat and respond in a constant, predictable manner to a given dose of extract per kilogram body weight. The suitability of the rabbit for prolan assay has been confirmed by Rowe, Simond and Nelson (1934). After the determination of the minimal ovulating dose of preg-

nancy urine extract in post partum rabbits, each injection of prolan into a group of pseudopregnant rabbits was check assayed within twenty-four to forty-eight hours in post partum rabbits to avoid any error that might arise from deterioration of the extract after dilution in distilled water. Thus the follicular sensitivity of the pseudopregnant group was in all instances determined in comparison to post-partum rabbits receiving the same extract at approximately the same time.

RESULTS. A. *Five day pseudopregnant rabbits.* At the dose level of one minimal ovulating dose of pregnancy urine extract, one rabbit was positive and six negative.

Five of the six negative rabbits showed some evidence of ovarian stimulation by the presence of either fresh corpora hemorrhagica[1] or cystic follicles.[2] At the dose level of two minimal ovulating doses, eight rabbits were positive and four negative and all the four negative rabbits showed evidence of some ovarian stimulation. At the dose level of three minimal ovulating doses, all six rabbits in this group were positive.

B. *Ten day pseudopregnant rabbits.* At the dose level of one minimal ovulating dose, one rabbit was positive and five negative. All five negative rabbits showed some evidence of ovarian stimulation. At the dose level of two minimal ovulating doses, all six rabbits in this group were positive.

C. *Fifteen day pseudopregnant rabbits.* At the dose level of one minimal ovulating dose, one rabbit was positive and six negative. Three of the six negative rabbits showed some evidence of ovarian stimulation. At the dose level of two minimal ovulating doses, all six rabbits in this group were positive.

It is evident from these results that at the fifth day of pseudopregnancy the ovary is one-half to one-third as sensitive to pregnancy urine extract as is that of the post partum rabbit, and at the tenth and fifteenth days of pseudopregnancy the ovary is no less than one-half as sensitive as the post-partum rabbit ovary. At the fifth day of pseudopregnancy three minimal ovulating doses will uniformly induce ovulation in the presence of active corpora lutea.

DISCUSSION. Previous workers, Wolfe (1931) Snyder and Wislocki (1931), Jares (1932), and Rowlands (1935) have shown that ovulation could be induced in pregnant and pseudopregnant rabbits by the administration of pituitary or pregnancy urine extracts. Snyder and Wislocki (1931) found that there was a progressive decrease in the amount of pregnancy urine extract necessary to provoke ovulation in the pregnant rabbit as the duration of the pregnancy increased. No satisfactory quan-

[1] Corpora hemorrhagica are large cystic follicles, two to five millimeters in diameter, containing frank hemorrhage evident on gross inspection.

[2] Cystic follicles are large clear follicles with a diameter two to three times that of follicles in the oestrous ovary.

titative studies on follicular sensitivity were presented, due to obvious difficulties in assay, with the exception of Rowlands (1935) who showed that approximately two to three times the amount of pregnancy urine extract or pituitary gland extract was necessary to induce ovulation in fifteen day and twenty-five day pregnant rabbits as was necessary to induce ovulation in oestrous rabbits. This finding is quite similar to the results of this study using pseudopregnant rabbits.

The development of ripe follicles in the presence of the corpus luteum of pregnancy has been described by Loeb (1911), Hammond (1927), Swezy and Evans (1930) and Cole, Howell and Hart (1931). These studies indicate that in the guinea pig, rat and cow, at least morphological maturation of the follicles does occur in the presence of a functioning corpus luteum, and in the mare that the follicles are morphologically and functionally mature during pregnancy.

The appearance of large follicles in the rabbit at the sixth day of pseudopregnancy, at which time an increasing number of rabbits will accept coitus (Hammond and Marshall, 1925), is correlated with an increase in follicular sensitivity to pregnancy urine extract. This suggests that sensitivity to pregnancy urine extract is indicative of the degree of follicular maturation present, and that after the fifth day of pseudopregnancy the follicle is only slightly less mature than during oestrus.

Hill (1934) determined by quantitative assay the amount of gonadotropic hormone present in the pituitary gland of the rabbit at various stages of pseudopregnancy. Following coitus in the oestrous rabbit there is almost complete discharge of all the gonadotropic hormone in the pituitary gland within twenty-four hours, so that the gonadotropic hormone content of the gland drops from an oestrous level of ten minimal ovulating units to one and one-half ovulating units. Hill (1934) calculated that at least four to five minimal ovulating doses of gonadotropic hormone are discharged by the stimulus of coitus for the induction of ovulation in the oestrous rabbit, a quantity far in excess of that necessary to provoke ovulation. On the tenth and fifteenth days of pseudopregnancy the gonadotropic hormone content of the pituitary gland rose to between twelve and twenty-one minimal ovulating doses. It is evident that the pituitary gland at these stages of pseudopregnancy contained more than sufficient gonadotropic hormone to induce ovulation, since at the tenth and fifteenth days of pseudopregnancy, two minimal ovulating doses of pregnancy urine extract uniformly produced ovulation.

Since this study has demonstrated that the follicles are capable of responding to the quantities of gonadotropic hormone present in the pituitary gland during pseudopregnancy, it must be concluded that some change associated with pseudopregnancy, probably the corpus luteum hormone, may act partially or completely to block the post-coital discharge of the pituitary gland and thus inhibit ovulation. If only partial block be pres-

ent, less than two minimal ovulating doses are liberated by coitus at the tenth and fifteenth days of pseudopregnancy.

Another mechanism of inhibition of ovulation following coitus during pseudopregnancy must be considered. There is the possibility, rather remote, that in pseudopregnancy the metabolism of the gonadotropic hormone may be so altered that even though it be released by the stimulus of coitus from the pituitary gland, the hormone might be inactivated or destroyed before reaching the follicle in sufficient concentration to provoke ovulation. One must postulate, however, that the gonadotropic hormone liberated from the pituitary in pseudopregancy would be metabolized in a different manner than pituitary extract or pregnancy urine extract injected into the pseudopregnant rabbit, since these substances have been demonstrated to provoke ovulation during pseudopregnancy.

SUMMARY AND CONCLUSIONS

1. The follicular sensitivity of the pseudopregnant rabbit to pregnancy urine extract is not less than one-third the oestrous level at the fifth day of pseudopregnancy, and not less than one-half the oestrous level at the tenth and fifteenth day of pseudopregnancy.

2. The follicles are quite able to respond by ovulation to the quantities of gonadotropic hormone found by Hill to be present in the pituitary at the tenth and fifteenth day of pseudopregnancy. The failure of the follicles to ovulate following coitus during pseudopregnancy suggests that some influence of pseudopregnancy, probably corpus luteum hormone, acts to produce a partial or complete failure of post-coital pituitary gland discharge.

We should like to take this opportunity to express our sincere appreciation for the advice and interest of Dr. Maurice H. Friedman during the course of this study.

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THE EFFECT OF PROGESTIN AND PROGESTERONE ON OVULATION IN THE RABBIT

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Received for publication January 12, 1937

It has long been considered that the secretion of the corpus luteum during the functional life of the lutein body was responsible for both the absence of heat and the cessation of ovulation. There has been, however, no satisfactory proof for this assumption. The following experiments were designed to determine whether or not progestin prevents ovulation in the rabbit, and if so, by what mechanism.

METHODS. All animals used in these experiments were healthy postpartum does, whose litters had been removed immediately after birth. They were all eating well and holding or gaining weight over the experimental period. The animals were kept in strict isolation in individual cages, beginning approximately one week before the estimated date for littering.

The progestin used was obtained from two sources. One portion was a relatively pure extract made from the corpora lutea of sows by the method of Allen and Goetsch (1), carrying the process through the first extraction with petroleum ether. The petroleum ether was distilled off and the residual oil dissolved and stored in methyl alcohol. The hormone, when used, was dissolved in commercial corn oil and assayed in terms of rabbit units (R.U.) by the method of Corner and Allen (2). The other portion of the progestin used was in the form of the crystalline hormone, progesterone,[1] synthesized from stigmasterol. This product is supplied in solution in sesame oil. One milligram of the hormone, equivalent to one International Unit, gave at least a four plus reaction when assayed by the Corner-Allen (2) method.

The progestin injections were given subcutaneously in the morning, between 9:00 and 10:00 a.m. daily. The amounts of progestin used were chosen with the work of Allen and Corner (4) in mind. These investiga-

[1] A generous portion of this material was supplied to us without charge, through the kindness and courtesy of Dr. Erwin Schwenk, of the Shering Corporation.

tors were able to carry to term rabbits, castrated shortly after coitus, by injecting one rabbit unit of progestin daily.

In some instances the does were mated. The attempt at mating was made, unless otherwise stated, in the late afternoon of the fifth day of injection, usually very close to 5:00 p.m. The usual signs of mating were checked in each case by an examination of the vaginal smear for sperm. In other instances, a highly purified and concentrated solution of Antuitrin S was injected intravenously.[2] This material was assayed on every occasion immediately before using, by the method described by Friedman (3).

EXPERIMENTAL. A. *Animals receiving two rabbit units of progestin or two milligrams of progesterone daily for five days.* Of the first group of 14 animals, 9 were injected with progestin and 5 with progesterone. An attempt was made to mate each animal at the end of the fifth day. Of the 14 animals, only 5 accepted the buck. The mated rabbits were opened 18 hours after coitus and in no case had ovulation occurred.

The animals which refused mating, nine in all, were injected immediately after their refusal with one minimal ovulating dose of pregnancy urine extract. Seven of the nine injected animals had ovulated when opened 18 hours later.

B. *Animals receiving one milligram of progesterone daily for five days.* Of this group of 7 rabbits, 3 accepted the buck. When opened, their ovaries showed no ovulation points and no signs of gonadotropic stimulation. The other four rabbits refused coitus. Two were not injected with an ovulating substance but merely opened 18 hours after the attempted mating. Their ovaries showed no sign whatsoever of gonadotropic stimulation. The remaining two of the four that refused mating were injected with one ovulating dose of the extract of pregnancy urine. One ovulated and the other showed subovulating stimulation of the ovaries: namely, thirteen cystic follicles.

C. *Animals receiving five milligrams of progesterone daily for five days.* In this group of 5 rabbits, one was mated. Inspection of its ovaries the following morning showed that ovulation had not taken place. The other 4 animals were injected with one ovulating dose of the gonadotropic preparation. Two had ovulated, whereas the other two had not.

D. *Animals receiving two milligrams of progesterone daily for five days.* An attempt was made to mate the 5 animals of this group on the afternoon of the fifth injected day. All refused the buck. They were not injected with the gonadotropic extract and the progestin injections were not continued beyond the fifth dose. Attempts were made to mate the rabbits both morning and afternoon of the sixth day, but unsuccessfully.

[2] This material was furnished to us gratis through the generosity of Dr. Oliver Kamm, of Parke Davis and Co.

On the morning of the seventh day following the first progesterone injection, the does were again placed with the buck and all five were successfully mated. At laparotomy, 24 hours later, it was found that every one of the five had ovulated normally.

Discussion. Many authors have produced evidence that the presence of a functional corpus luteum prevents spontaneous ovulation in most mammalian species (7, 8, 9, 10, 11, 12), the one exception being the horse (5). The rabbit, which normally ovulates after coitus, will accept the buck during pregnancy and pseudopregnancy but ovulation does not occur (6). It cannot be doubted, therefore, that the presence of a functional corpus luteum is associated with some interference in the progression of those ovarian changes which ordinarily culminate in ovulation.

Whether or not this is effected by the corpus luteum hormone directly is another question. Numerous attempts have been made to suspend ovulation or change the rhythm of the sexual cycle in various animals by injecting corpus luteum material. Success in this direction was reported by many investigators, (8, 13, 14, 15, 16, 17, 18, 19), who employed crude extracts of doubtful potency. More recent investigators have reported the inhibition of the oestrous cycle and ovulation in rats (20, 21, 22), in mice (23, 24) and in guinea pigs (25, 26). These recent investigators employed definitely potent and in some cases chemically pure preparations. It is also possible that Corner (27) prevented ovulation in the monkey by the injection of one rabbit unit of progestin daily.[4]

Granting, however, that the corpus luteum hormone is able to suspend ovulation and oestrus, the mechanism through which this suspension is effected remains to be explained. It is obvious that the corpus luteum hormone could inhibit ovulation by: 1, decreasing the sensitivity of the follicle; 2, by interfering with the release of the gonadotropic hormone of the pituitary, or 3, by destroying or altering the hormone in the blood stream by some indirect metabolic process.

The destruction of the gonadotropic hormone by some metabolic process peculiar to pregnancy or pseudopregnancy seems highly improbable in view of the fact that *any* kind of gonadotropic preparation, given intravenously in doses not much greater than those necessary to ovulate an oestrous rabbit, can induce ovulation in the presence of functional corpora lutea in the ovaries (Weinstein, 28).

The experiments reported in this paper indicate clearly that the follicular sensitivity to pregnancy urine extract is not decreased by the corpus luteum hormone in the doses employed. It may be seen that of the 15 animals receiving corpus luteum injections, 10 ovulated when one mini-

[4] While this paper was in press our attention was brought to the work of Mahnert in the Zbl Gynäkol. 54: 2883, 1930 who reports the failure of post-coital ovulation in isolated rabbits after the injection of corpus luteum extract.

mum ovulating dose of Antuitrin S was injected. In view of the closeness of our original assay of the Antuitrin S, it seems fair to attribute the failure of ovulation in the remaining 5 animals to the unvoidable biological variation of the animals and not to any effect of the corpus luteum hormone which had been injected. In this connection, it is pertinent to note that three of these five animals which had failed to ovulate showed signs of some ovarian stimulation, as attested by the presence of cystic follicles in the ovaries.

The results of the matings of the corpus luteum injected animals lend strong support to the second possibility mentioned above. It may be seen from our data that ovulation failed to occur following coitus in every one of the 9 rabbits that had received one or more rabbit units of a corpus luteum preparation daily for 5 days.[3] When one considers the regularity of post-coital ovulation in post-partum rabbits (6), the failure of ovulation in the post-partum rabbit injected with progestin must be attributed either to an interference in the post-coital release of hormone from the pituitary, or to a supression of the process of synthesis and storage of the hormone in the pituitary during the progestin injection. This latter possibility cannot be considered seriously since the secretory activity of normal corpora lutea does not prevent the synthesis and storage of gonadotropic hormone in the pituitary in amounts at least equal to that found in an oestrous rabbit, and more than adequate to cause ovulation if released (29, 30).

There remains, therefore, only one likely mechanism of the progestin inhibition of ovulation: that is, some interference with the post-coital release of the pituitary hormone.

SUMMARY

1. Both an extract of sow's corpora lutea and progesterone, prepared from stigmasterol, inhibit ovulation after mating in the rabbit.

2. This inhibition does not extend more than 48 hours after the last injection of the hormone.

3. Many of the post-partum (oestrous) rabbits were thrown out of heat by the injections and would not accept the buck.

4. In rabbits receiving a corpus luteum preparation in doses equal to or greater than amounts adequate to sustain pregnancy, the ovarian follicles will ovulate in response to a minimal ovulating dose of a gonadotropic preparation.

5. The action of progesterone, as disclosed in these experiments, is sufficient to explain the inhibition of ovulation during pregnancy and pseudo-

[3] One rabbit received 0.2 mgm. of progesterone per day, for 5 days and ovulated after mating on the fifth day. The dose was below the threshold necessary to inhibit ovulation.

pregnancy in the rabbit. This inhibition is not exercised directly upon the ovarian follicles, but at a point more central in the ovulation provoking mechanism.

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PROPRIOCEPTIVE RESPIRATORY REFLEXES OF THE VAGUS NERVE[1,2]

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Received for publication January 13, 1937

The first theory of the respiratory function of the vagus nerve to attract lasting attention was the logical schema of Hering and Breuer (1868) according to which inspiration is inhibited and each succeeding expiration is provoked by stimulation of the inspiratory inhibitory fibers, and expiration is inhibited and each succeeding inspiration is evoked by stimulation of the expiratory inhibitory fibers. Strangely enough the very completeness of this arrangement for the speeding up and control of the automatic rhythm of the central respiratory mechanism served as the first important point of attack when Gad in 1880 advanced a simpler theory requiring the action of the inspiratory inhibitory fibers alone. The next important contribution was that of Head (1889), which combined the ideas of Hering and Breuer and those of Gad. Though stressing the predominant rôle of the inspiratory inhibitory fibers in the control of breathing, the rôle of the expiratory inhibitory fibers was not entirely dismissed. The question of vagal function had thus resolved itself largely to the determination of the existence of expiratory inhibitory fibers and to the relative importance of the two groups of fibers should they exist—see Schenk (1903, 1905), von Lewandowsky (1898), Alcock and Seeman (1905), Einthoven (1908), Keller and Loeser (1930), Adrian (1933), Partridge (1933, 1935).

Recently another point of view has been advanced by Hammouda and Wilson (1932, 1935a, b) who studied the question of vagal function by a combined procedure of graded vagal block plus alternate inflation and deflation of the lungs. They described a disappearance of reflex slowing of breathing from pulmonary inflation at vagal block temperatures of approximately 8°C. and a continued persistence of acceleration from pulmonary deflation down to 0°C. This apparent *differential block* of inhibitory and acceleratory effects convinced them of the existence of two separate and distinct reflexes, *inhibitory* from inflation and *acceleratory* from collapse. The further fact that acceleration was obtained with lung inflation as well as with deflation at the lower block temperatures, led them to believe that

[1] Preliminary report, This Journal Proc. **116:** 17, 1936.

[2] This study was supported in part by a grant from the Rockefeller Foundation.

the acceleratory fibers are stimulated by inflation as well as deflation, just as Adrian (1933) claimed for the inspiratory inhibitory fibers.

If we understand Hammouda and Wilson correctly, a relatively important issue is involved, for they seem to imply that a continuous excitation of a certain group of proprioceptive fibers provokes acceleration similar to that commonly observed from prolonged faradic stimulation of a sensory nerve where the element of pain is probably predominant. Such a view differs from the classical conceptions of vagal control according to which acceleration is accomplished through a series of rhythmic reflexes set up by volume changes of the lungs. The idea of a distinct acceleratory reflex additional to and differing from those described by Hering and Breuer gained support from the experiments of Gesell and Moyer (1935) who showed that the effects of intravenous injection of Na_2CO_3 on breathing varied with the degree of distention of the lungs. When the lungs were inflated breathing was further retarded, but when they were deflated breathing was frequently further accelerated. Since carbonate augments prevailing reflexes the results suggested the existence of two distinct proprioceptive reflexes, an inhibitory reflex set up by inflation and an acceleratory reflex set up by deflation. More recent experiments (Brookhart, Steffensen and Gesell, 1936), however, offer another explanation and suggest that the reflex acceleration which was augmented by carbonate injection was initiated by excitation of pain endings rather than by stimulation of specific acceleratory fibers.[3]

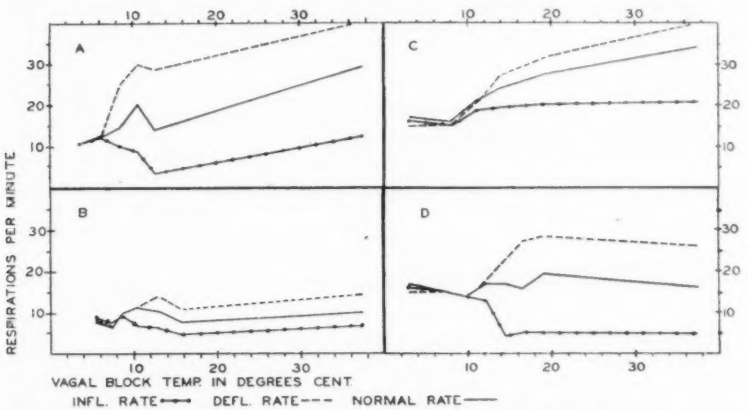
If the assumption be made that there are several sets of proprioceptive fibers passing from the lungs in the vagus nerves there is evidence enough to indicate that a functional identification of fibers might be possible by means of differential blocking. Head (1889) described a paradoxical acceleration of breathing from pulmonary inflation during a certain stage of recovery from freezing of the vagus nerves which was confirmed by Hammouda and Wilson. Fränkel and Gad (1889) obtained a differential cold block of the recurrent laryngeal nerve, the fibers to the abductor muscles blocking out earlier than those to the adductor muscles. Howell, Budgett and Leonard (1894) found in the cat and rabbit that central stimulation of the vagi at temperatures above 7°C. elicited acceleration which effect was blocked out at lower temperatures. The acceleration was attributed to pain.

Meek and Leaper (1910) employing their pressure blocking device on nerve found that the degree of block depended on the amount and duration of pressure and revealed the possibilities of graded effects. Gasser and Erlanger (1928) gained evidence that nerve fibers of large diameter are

[3] There is no implication in this statement that any of the animals suffered pain during the course of our experiments. Anesthesia was always maintained at a high enough plane to avoid such effects.

blocked more quickly than fibers of small diameter. Heinbecker and O'Leary (1933) found that of the functions mediated by myelinated fibers the afferent fibers serving respiration are larger than those serving the vasomotor center and may be differentially blocked, the respiratory fibers yielding first. The authors made no attempt to establish the identity of functional types of respiratory fibers.

Accordingly we attempted to study the question of plurality of function of the vagus nerve by the selective action of graded interruption of vagal impulses with cold and pressure block on such reflexes as are set up by inflation and deflation of the lungs with the method described by Gesell and Moyer (1935). As a control we used graded sectioning of the nerve on the assumption that such procedure could exert a differential effect only



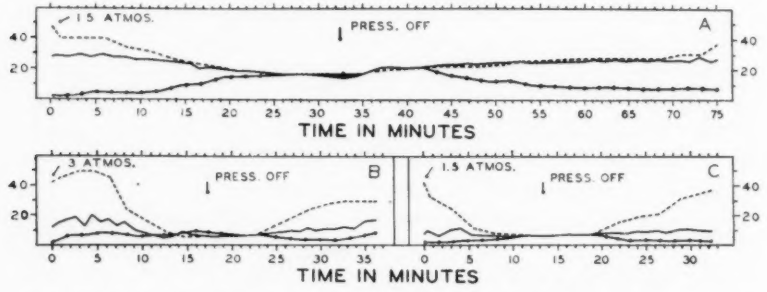
Figs. 1A, B, C and D. Effects of graded cold block of the vagus nerves on the reflex response to inflation and deflation of the lungs. The respiratory rates are plotted on the ordinates against temperature of the vagal block on the abscissae. The curves read from right to left.

through the chance of selective division of one of the hypothetical groups of fibers.

Results. I. *Effects of graded temperature block on vagal function.* There were sixteen experiments on cold block from which four observations are shown in figures 1A, B, C, and D. In six of these the dogs were anesthetized with urethane and morphine, in eight, with barbital and two were decerebrated during evanescent anesthesia (evipal). The nerves were completely and snugly surrounded for a length of 4.5 cm. with thin copper plate exposed internally to circulating water from a large reservoir and protected externally from loss of cold by cork insulation. Specially mounted thermometers permitted precise control of blocking temperatures.

Inspection of figures 1A, B and D shows that acceleration due to defla-

tion was approximately equal to the slowing due to pulmonary inflation with the vagus nerves at body temperature. The negative and positive pressures were always equal. As the nerves were progressively chilled there was an approximately equal reduction of the inhibition and acceleration of the rate of breathing and an approximately symmetrical convergence of the respiratory rate curves, which would indicate that the two reflex responses were equally affected by a reduction in the number of conducting fibers. In figure 1C the initial acceleration due to lung deflation was less than the slowing due to inflation. This is a fairly common finding and will be considered again in relation to types of anesthesia. Where such initial disproportionate responses to deflation and inflation occurred progressive vagal block also produced the same symmetrical convergence as seen in figures 1A and 1D. An occasional peculiar response,



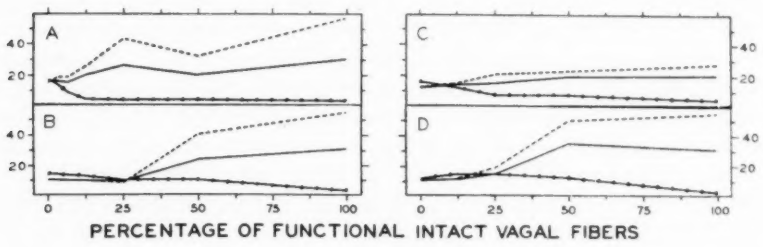
Figs. 2A, B and C. Effects of graded pressure block of the vagus nerves on the reflex response to inflation and deflation of the lungs. The respiratory rates are plotted on the ordinates against time on the abscissae. The observations in 2A were made under morphine urethane anesthesia and those in 2B and C under barbital anesthesia. Broken line—deflation rate; continuous line—normal rate; beaded line—inflation rate.

which remains to be explained, is illustrated in the upward swing of all the curves just prior to convergence in figure 1B. The occasional coincidence of two curves and sporadic variation common to all of our findings were probably due to the slightly unsteady states of the animals.

II. *Effects of graded pressure block on vagal function.* In studying the effects of pressure block the method of Meek and Leaper (1910) was modified to permit the application of pressure to an intact nerve. Each vagus nerve was placed in a groove of a semicylinder of brass 65 mm. in length and a ligated artery lying in a groove of a superposed and tightly fitting second semicylinder was connected with a gas cylinder to provide the blocking pressure. Experience showed that a pressure of approximately 1000 mm. Hg produced a gradually developing block satisfactory for our studies.

Five experiments were done, three on dogs under urethane-morphine anesthesia and two under sodium barbital anesthesia. It will be seen that as the pressure continued the effects of deflation and inflation progressively diminished and that complete convergence occurred at intervals varying from fifteen to thirty-five minutes. With the release of pressure, conduction returned at about the rate at which it disappeared, a rather interesting observation in itself. In figure 2A the slowing due to lung inflation was greatly in excess of the acceleration from deflation, a not uncommon finding in dogs anesthetized with morphine and urethane. In figures 2B and 2C the acceleration due to deflation was greatly in excess of the inhibition from inflation, a result not uncommon to barbital anesthesia.

III. *Effects of progressive section of the vagus nerves on vagal function.* Five experiments were conducted, three under urethane morphine anesthesia and two under sodium barbital anesthesia. One nerve at a time was progressively sectioned and in preparation for that procedure the sheath was very carefully removed to avoid injury to the conducting fibers. The other nerve was either completely sectioned or cold blocked, thus reducing the conducting fibers to one-half. Deflation and inflation reflexes were then elicited following which the desheathed nerve was hemisectioned, reducing the fibers by another half. In this fashion, the number of functioning fibers was reduced by one-half with each section until the strand of nerve was too small for further hemisection. For purposes of precision, hemisection was conducted under a binocular dissecting microscope. The respiratory rates were then plotted on the ordinates against the percentage of intact fibers on the abscissae as illustrated in figures 3A, B, C, and D.



Figs. 3A, B, C and D. Effects of graded section of vagal fibers on the reflex response to inflation and deflation of the lungs. The respiratory rates are plotted on the ordinates against the percentage of intact vagal fibers on the abscissae. For further explanation see text. Broken line—deflation rate; continuous line—normal rate; beaded line—inflation rate.

The effects of section of vagal fibers resembled those of temperature and

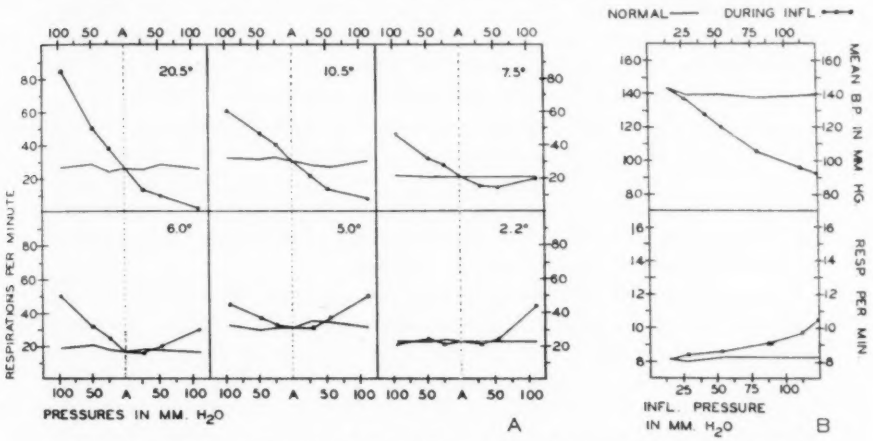
pressure block. Convergence increased with decreasing number of conducting fibers and occurred respectively at 1, 3, 5, 5, and 22 per cent respectively of residual fibers. In graph 3A when the last remaining strand was barely visible inflation and deflation still produced definite slowing and acceleration of breathing. In graphs 3B and D obtained from successive section of the left and right vagus nerves respectively (during the first graded section the right vagus was cold blocked) the percentage of intact vagal fibers at convergence was for some unknown reason exceptionally high. Comparison of 3D with the overlying graph 3C illustrates again the disproportionately greater acceleration of breathing produced by deflation during barbital anesthesia as compared with that during morphine-urethane anesthesia.

The causes of these peculiar effects of urethane-morphine and of barbital anesthesia on the reflexes initiated by deflation and inflation of the lungs have not been determined. It is possible that there is a selective action of these drugs on the proprioceptive endings in the center or lungs or upon the expiratory position of the chest (Adrian, 1933). Moyer and Gesell (1935) have called attention to the possible rôle of expiratory lung volume in the reflex control of breathing, and granting that barbital produces a large chest volume during the expiratory pause, the resulting continuous firing of the vagi would inhibit the rate of breathing. Under such circumstances deflation of the lungs should produce greater acceleration of breathing when the lung volume is large than when the volume of the lungs is smaller.

After convergence or approximate convergence of the respiratory rate curves, regardless of the method employed to impair vagal conduction, the animal still responded to deflation and inflation of the lungs but with variable results. The so-called reversal effects of Hammouda and Wilson were common, but, on the whole, the effects of inflation and deflation were small as compared with those obtained at the higher temperatures. They gave the appearance of being a resultant dependent on minor interacting components. Hammouda and Wilson, however, laid particular stress on their reversal effects (see fig. 1B) obtained at a vagal block temperature of 6°C. at which point they believed the inhibitory fibers to be selectively blocked thus permitting full acceleration from stimulation of the acceleratory fibers by either deflation or inflation of the lungs.

By subjecting the lungs to a series of graded positive and negative pressures at a number of temperatures, between normal and 0°C. (see fig. 4A) we were able to analyze such reversal effects in greater detail. The first graph shows the reflex acceleration and inhibition at a vagal temperature of 20.5°C. Lowering the temperature to 10.5°C. produced a slight reduction of the response to inflation and deflation. There is little change if any in the shape of the curves, the acceleratory and inhibitory response being

proportionately reduced. At 7.5°C. the acceleration with deflation is again decreased, but slowing from inflation was disproportionately reduced. As a matter of fact the right portion of the curve bends upward as the inflation pressure increases, and at the greatest inflation there is practically no slowing of breathing. This bending of the curve indicates the operation of two effects of inflation, one of slowing, coming from stimulation of the pulmonary vagal inhibitory endings, and the other of acceleration coming from some unknown source; furthermore, it shows that the accel-



Figs. 4A and B. Reversal effects of increased pulmonary pressure and their explanation.

4A. A series of responses to graded pulmonary inflation and deflation at 20.5, 10.5, 7.5, 6.0, 5.0, and 2.2°C. vagal block temperatures. The normal respiratory rate (continuous line) and the response to graded pulmonary pressures (beaded line) are plotted on the ordinates against decreased intrapulmonary pressures to the left and increased intrapulmonary pressures to the right of atmospheric pressure A. For a description of results see text.

4B. The relation of reversal effects to changes in mean blood pressure after double vagotomy. The lower graph shows increasing respiratory rates with increasing pulmonary pressures. The upper graph shows the relation of respiratory rates to the accompanying decrease in mean blood pressure.

erating effect grows more rapidly with progressive inflation than the inhibitory effect. At a slightly lower vagal temperature (6°C.) the typical reversal effects of Hammouda and Wilson appear. Both inflation and deflation produce acceleration, and acceleration increases with rising inflation. At 5°C. the acceleration due to deflation is markedly reduced and the acceleration of inflation is increased even more. At 2.2°C. acceleration from deflation is completely missing and acceleration from progressive lung inflation is at its maximum. The argument of Hammouda and

Wilson holds at 7.5, 6.0 and 5.0°C., but if this acceleration with positive pressure is due to the stimulation of acceleratory fibers, what is the stimulus to acceleration from inflation at 2.2°C., where deflation fails to augment breathing and where we may assume complete blockage of all vagus fibers? This acceleration seems to be related to the drop of blood pressure produced by pulmonary inflation, for when the positive pressure was sufficient to cause an appreciable drop in blood pressure, graded positive pressures causing graded drops in blood pressure could be correlated with graded reversal effects (see fig. 4B). In four experiments reversals were obtained after bilateral vagotomy. In each of these cases, the reversals were abolished by occlusion of the carotid arteries, which indicates that the carotid sinus region is the reflex source of the accelerated breathing.

It is likely that similar changes in blood pressure also influence respiratory response with the vagi functioning. Such effects as well as unsteady states of the animal, therefore, must contribute to the irregularities, imperfect convergences and sporadic coincidence of the respiratory rate curves which were noted before, but the main factor shaping the rate curves seems to be the vagal proprioceptive reflexes.

Since our experiments fail to confirm an acceleratory group of fibers said to be stimulated by both inflation and deflation of the lungs, we may continue to consider the respiratory function of the pulmonary vagal fibers from the standpoint of the proprioceptive groupings proposed by Hering and Breuer and by Gad and by Head. Briefly summarized, our data obtained with all three methods of interruption of vagal conduction indicate that the vagal influence on rate of breathing is closely dependent upon the number of fibers conducting, or rather the number of proprioceptive impulses reaching the center; and that the type of response to inflation and deflation of the lungs, as indicated by the rate analysis, is unaltered by the number of fibers conducting. The fact that two procedures potentially capable of selectively interrupting nerve fibers of different physiological action and a third control procedure incapable of such selectivity yielded similar results, is suggestive, but cannot be interpreted as positively indicating that vagal function is exercised through only one set of vagal proprioceptive fibers. It is conceivable that two closely related reflexes might have afferent fibers of closely similar blocking characteristics despite our introductory support of the experimental possibilities of differential blocking. In the second place the proprioceptive groups of vagal fibers as described by Hering and Breuer have a reciprocal relation working towards similar changes in rate of breathing. A differential blocking might, therefore, be completely missed with rate analysis alone. The most that one may postulate at present is that the inspiratory inhibitory group of fibers exert a predominant control of breathing. Such an assumption allows

simpler explanations of various respiratory phenomena, but the possibility of their incompleteness must not be overlooked.

SUMMARY AND CONCLUSIONS

The respiratory functions of the vagus nerve were studied with special reference to the number of proprioceptive reflexes contributing towards the control of the rate of breathing.

The procedure employed was to note the effects of selective block (progressive cold and pressure) and of non-selective interruption of vagal conduction (progressive section) on the changes of the rate of breathing reflexly elicited by inflation and deflation of the lungs.

Progressive interruption, regardless of the methods employed, reduced the slowing from inflation and the acceleration from deflation and produced an approximately symmetrical convergence of the inflation, deflation and normal respiratory rates. This emphasizes the importance of the number of signals arriving at the central mechanism.

Final convergence occurred at about 5°C., at about 25 minutes of 1000 mm. Hg pressure and at about 3 per cent uncut vagal fibers.

After convergence and also after complete double vagotomy inflation of the lungs still accelerated the rate of breathing, thus demonstrating the extra-vagal source of the respiratory acceleration and of the reversal phenomenon described by Head, and Hammouda and Wilson. These reversal effects varied in degree with the magnitude of lung inflation and were roughly proportional to an accompanying fall in blood pressure. They appeared to arise in the carotid sinus region for they were eliminated by clamping of the carotids.

It is accordingly suggested that an accessory mechanism, whereby breathing is accelerated by a continuous stimulation of a special group of pulmonary proprioceptive fibers may not exist.

The absence of differential effects during differential vagal block agrees with exclusive or predominant control of breathing by the inspiratory inhibitory fibers but does not positively eliminate the possibility of additional control by the expiratory inhibitory fibers.

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IS THE TRANSMISSION OF THE NERVE IMPULSES TO THE CAT'S UTERUS ELECTRICAL OR CHEMICAL?

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Received for publication January 18, 1937

Piperidinomethylbenzodioxane (933F) diminishes only slightly the responses of various organs and systems to nervous excitation, while markedly decreasing responses to adrenalin and sympathin. This selective effect has been demonstrated for the nictitating membrane by Bacq and Fredericq (1935a and b), for the spleen by Heymans and Bouckaert (1935), and for the vasomotor reflexes by Vleeschhouwer (1935). We have attempted to determine whether or not 933F exerts a similar differential effect upon the responses of the cat's uterus to nervous excitation and adrenalin.

METHOD. Pregnant and non-pregnant cats were used under dial anesthesia (0.8 cc. per kgm.). Both adrenal glands were ligated. Artificial respiration was employed to prevent the development of asphyxia. Both hypogastric nerves were isolated immediately distal to the inferior mesenteric ganglia, and placed on shielded silver electrodes. The nerve trunks proximal to the electrodes were crushed. With the secondary coil of a Harvard inductorium at 7 cm. and a primary current of 7 volts, faradic stimuli of 10 to 15 seconds' duration were employed. Injections of adrenalin in dosages varying from 0.3 γ to 20 γ were made into the femoral vein. Isotonic contractions of the uterine muscle were recorded before and after intravenous administration of 933F (3 to 10 mgm. per kgm.).

Histological examination of the ovaries and uterine endometrium were made after the experiments to determine the presence or absence of corpora lutea and decidual reaction.

RESULTS. 1. *Non-pregnant animals.* In certain non-pregnant animals the relaxation caused by stimulation of the hypogastric nerves was preceded by a preliminary contraction (fig. 1A). Injections of adrenalin caused relaxation alone (fig. 1B). These observations are in accord with those of Cushny (1906) and Dale (1906) who also noted a preliminary small contraction and subsequent relaxation as a result of stimulation of the hypogastric nerves in several non-pregnant cats. These investigators attributed the phenomenon to incomplete involution of the pregnant uterus, and concluded that stimulation of the "true non-pregnant" uterus

by either nervous excitation or adrenalin caused relaxation, while similar stimuli applied to the pregnant uterus caused contraction. Histological examination of the ovaries of our animals in which the uteri gave the preliminary contraction showed the presence of one or more small corpora lutea, although the uterine endometrium showed no evidence of decidual response or secretory activity. Evans and Swezy (1931) have stated that ovulation and corpus luteum development may occur in the cat without

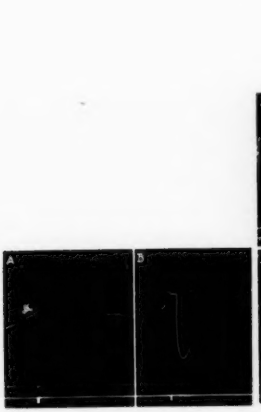

Fig. 1

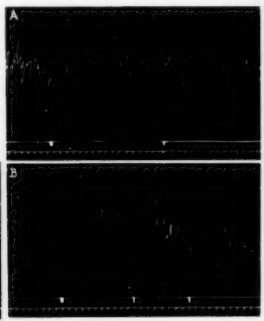

Fig. 2

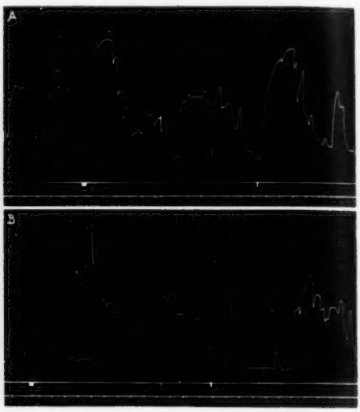

Fig. 3

Fig. 1. Responses of the uterus of a non-pregnant cat, A to tetanic stimulation of the hypogastric nerves for 15 seconds, B to an intravenous injection of adrenalin (0.2 γ). In this and the succeeding figures the time signal records 30 second intervals.

Fig. 2. Non-pregnant uterus.

A. Before 933F. Responses to stimulation of the hypogastric nerves (15 sec., coil distance 7 cm.) and to adrenalin (0.5 γ).

B. After injecting 4 mgm. per kgm. of 933F. Responses to hypograstric stimulation (as in A), and to adrenalin (0.5 and 1.5 γ).

Fig. 3. Pregnant uterus.

A. Before 933F. Responses to hypogastric stimulation (10 sec., coil distance 7 cm.) and to adrenalin (10 γ).

B. After injecting 1 mgm. per kgm. of 933F. Responses to hypogastric stimulation and adrenalin as in A.

copulation. That the reversal of the response of the uterus to sympathetic stimulation during pregnancy is due to the corpus luteum hormone has been shown by Van Dyke and Gustavson (1929), Gustavson and Van Dyke (1931), and Kennard (1937). It is suggested that the secretion of the corpus luteum of the non-pregnant animal may cause the initial contraction described, although the hormone may not be present in quantity sufficient to bring about complete reversal of response. These

observations have been investigated by Kennard (1937), who has offered a tentative explanation of the differential effect of nervous excitation and adrenalin.

The effects of 933F are illustrated in figure 2. Before administration of 933F both hypogastric stimulation and adrenalin injection caused a marked relaxation of the uterus, which in some instances was preceded by a small initial contraction, as reported above. Following 933F, nerve stimulation caused a relaxation of magnitude only slightly less than that previously observed; the initial contraction was also slightly decreased in magnitude. The response to adrenalin was very markedly decreased, however—dosages ten times the quantity previously used being required to cause relaxation of comparable magnitude.

2. *Pregnant animals.* Hypogastric stimulation and adrenalin injections caused uterine contraction. Following administration of 933F the response to the nerve impulses was unaffected or only slightly decreased, while the contraction resulting from injection of adrenalin was greatly decreased (fig. 3). A period of inhibition followed the contraction to adrenalin, during which spontaneous contractions were decreased in magnitude. This inhibition was accentuated by the injection of 933F.

Discussion. The differential effect of 933F on the responses of the nictitating membrane of the cat to stimulation by the cervical sympathetic and adrenalin has been the basis of Monnier and Bacq's (1935) theory that there exist both electrical and chemical transmission of nerve impulses to smooth muscle. They concluded that both modes of transmission were present in muscles showing a spike action-potential preceding contraction, and excitable by a single nerve volley, citing the nictitating membrane as an example. They postulated chemical transmission alone, on the other hand, in muscles responsive only to repeated nerve volleys, and showing no spike action potentials, citing the pregnant uterus of the cat as an example of this group. If their argument were valid 933F would exert an effect upon the responses of the pregnant cat's uterus equivalent to hypogastric nerve stimulation and to adrenalin. The experimental evidence, however, reveals that 933F exerts a differential effect upon the action of nerve stimulation and adrenalin on the pregnant cat's uterus (fig. 2) in a manner identical with that observed in the nictitating membrane. This evidence is contrary to the assumptions of Monnier and Bacq.

The observations of differential action upon nerve stimulation and adrenalin in an organ in which spike potentials have not been found (Rosenblueth, Leese and Lambert, 1933), requires an explanation different in nature from that of Monnier and Bacq. Such an explanation should account for the effects observed on the nictitating membrane, the spleen and the vascular system, as well as those described here for the uterus. It should also account for the fact that the depressant action of 933F is not

specific for adrenalin and sympathin, but also extends to other substances as well (Vleeschhouwer, 1935; Ross, 1936).

Rosenblueth and Cannon (1936) cite facts suggesting that 933F increases the polarization of the cell membrane, thereby making it less permeable to adrenine and decreasing the responses to this agent. Sympathin, liberated intracellularly as a result of nerve stimulation, would be unaffected in its local action, although diffusion to non-innervated cells would be decreased, with some diminution in total response. Such a hypothesis explains the observed facts and makes it unnecessary to postulate both electrical and chemical transmission of the nerve impulse to smooth muscle.

SUMMARY

The responses of the non-pregnant and pregnant uterus of the cat to adrenalin are decreased by 933F, while responses to stimulation of the hypogastric nerves are relatively unaffected (figs. 2 and 3). These results are discussed in relation to the theories of transmission of the nerve impulse to smooth muscle.

An initial contraction preceding relaxation was observed in the non-pregnant uterus in several cats as a result of nerve stimulation, although adrenalin caused relaxation alone (fig. 1). One or more small corpora lutea were found in the ovaries of these animals, although there was no evidence of pregnancy in the uterine endometria. A possible explanation of this observation is offered.

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THE COURSE OF BLOOD SUGAR AFTER INTRAVENOUS INSULIN IN NORMAL DOGS AND CATS[1]

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Received for publication January 18, 1937

In order to interpret certain other observations we felt the need of more data on the action of intravenous insulin in normal animals than are at present available. This paper undertakes to cover some of the rather elementary ground of determining over a reasonable dose range the effect of intravenous insulin in normal dogs and cats.

The very characteristic curve following intravenous insulin in the normal dog has been described (1, 2). Here we wish to go into more detail including data on the cat and discuss the possible significance of some of the features of the curve resulting when blood sugar values after various doses of insulin are recorded at intervals of about 15 minutes until return to initial values has taken place. Shorter intervals have been used in those portions of the curves which were of particular interest.

Several species including the human and the dog show a distinctly different blood sugar curve with intravenous and subcutaneous injection while in others, as indicated by Cannon's (3) work on the cat, no particular quality of response characterizes the mode of injection.

Observations concerned with the constancy of blood sugar under conditions where there is no known outside interference have resulted in the concept of the oscillation of the blood sugar. This is illustrated by the exhaustive studies of Hansen (4) on human subjects which have been verified by Albritton (5) on dogs. The up and down movement of usually about 10 mgm. occasionally reaching 30 mgm., occupies 10 to 20 minutes. After feeding of carbohydrates or after insulin the oscillations usually persist but with some modifications. Studies of blood sugar changes should, therefore, for maximum validity, be based on short intervals, say 3 to 5 minutes. Not in all cases, particularly when the blood sugar is undergoing marked change, will a curve with wider intervals represent the true picture, but Hansen's data indicate that for many purposes the 15 minute intervals

[1] This work has been made possible by a grant in aid from Eli Lilly & Co. We also gratefully acknowledge liberal supplies of insulin and amytal.

afford a sufficient approximation. The conclusions here reported take into consideration the presence of the oscillations.

Experiments on dogs with injection of 0.1 unit, 0.5 unit, and 1 unit per kilo intravenously. All animals were without food for 18 hours. Unless otherwise stated the ordinary insulin (Lilly) for clinical purposes was used. Injection was made into the jugular vein. A sample of U 10 insulin was in each case diluted so that the proper dose was contained in 1 cc. of solution. Blood samples were also drawn from the jugular vein. Sodium fluoride was used as anticoagulant. Blood sugar determinations were made by the Shaffer Hartman method using reagent I as given by Peters and Van Slyke (6, p. 466). Frequent determinations of non-fermentable reducing substance were carried out according to Somogyi (7). Only slight vari-

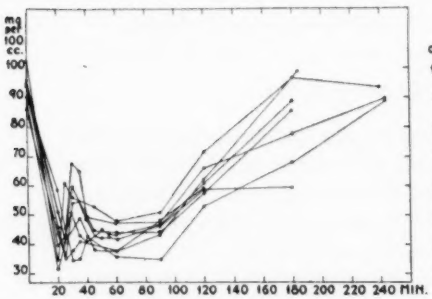


Fig. 1

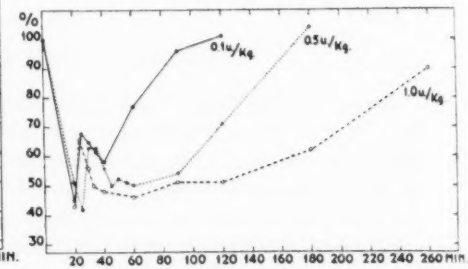


Fig. 2

Fig. 1. Seven individual blood sugar curves with 0.5 unit per kilogram of insulin in normal dogs. Blood sugar is given in milligrams per 100 cc. of blood.

Fig. 2. Effects of 0.1 unit, 0.5 unit and 1.0 unit of insulin per kilogram in normal dogs. Blood sugar is recorded as per cent of the initial value. Each curve represents a typical individual experiment. The variation in individual observations is given in the text.

ations were found in the same animal from time to time and, as noted by others, insulin injection did not modify the values.

The number of insulin experiments together with the properties characteristic of each dose are given in the text and typical curves are shown in figure 1. In figures 1 and 4 the results are recorded in milligrams (total reducing substance as glucose) per 100 cc. of blood while figures 2 and 3 indicate the change in relation to the initial value as 100 per cent. It is not practical to give average curves except for certain purposes. In dogs particularly, distortion will result because, while very similar in contour, portions of the curve may shift anywhere up to 10 minutes on the time axis (cf. Hansen's duplicates on both ears). For this reason the intermediate rise in the curve for the dog may almost disappear if results are averaged in spite of the fact that each individual curve contains a definite intermediate

rise. (Compare fig. 1 with fig. 1 of the following paper (8, p. 541.) Because the sharp reversal at about 20 minutes differentiates the intravenous insulin action in normal dogs so definitely from the subcutaneous action and also from that in depancreatized dogs (Zucker and Berg, 9), we have determined a larger number of values at shorter intervals in this region. Data so far obtained indicate that a large intermediate rise follows the precipitate drop and later on oscillations occur not necessarily larger than with resting blood sugar until sugar has returned to its original level (unpublished data).

One-tenth unit per kilogram. Twenty-two dogs showed the following characteristics: The initial drop to 40 per cent or 50 per cent of the original value lasted for 20 or sometimes 25 minutes. Between 20 and 40 minutes there was a temporary rise of from 15 to 30 mgm. followed by a drop usually about half as large as the rise. From 40 minutes on there was, judging by 15 minute intervals, a rather continuous rise back to normal, reaching this value 60 to 90 minutes after the injection.

Seven additional dogs on whom curves were obtained with 0.1 unit per kilogram gave atypical curves. These animals were found to be at the time of the experiments in the incubation period of distemper and showed symptoms from two to twelve days later. The curves are characterized by the absence of a reversal at 20 minutes. The minimum was deferred to 40 minutes or more and was followed by a much more gradual rise. Marked effects of active infectious processes on insulin action in laboratory animals have been described (Lawrence and Buckley, 10) and are also well known in humans. The minor effects here noted during the incubation period are of interest and emphasize the necessity of observing the general condition of the animals if consistent results are to be obtained.

Five-tenth unit per kilogram. Eight dogs. In time and extent of initial lowering results were the same as with 0.1 unit per kilogram. The peak of the intermediate rise came between 25 and 40 minutes. After the intermediate rise low values occurred between 45 and 90 minutes after which a fairly continuous ascent brought the level back to normal between 180 and 240 minutes after injection.

One unit per kilogram. Seven dogs. Again the initial drop was identical with that for 0.1 unit per kilogram. After the intermediate rise a blood sugar lowering almost reaching the previous minimum may continue up to 120 minutes. Then the curve rose, regaining the initial level at 240 to 300 minutes.

Over the dose range studied the initial drop in both time and extent of lowering shows that 0.1 unit per kilogram gives maximal effects. With regard to duration of action, however, 0.1 unit per kilogram does not give maximal effects. These results are similar to those of Bodansky (11) on sheep and differ from those reported for cats below.

In view of the findings of Geiling and de Lawder (12) on the effects of impurities associated with insulin, we repeated our observations with a preparation of purified but non-crystalline insulin kindly supplied by Eli Lilly Company which was rated at 22 to 24 units per milligram, a value within about 10 per cent of the maximum activity attributed to crystalline insulin. Ordinary insulin for clinical use has about 20 units per milligram. Crystalline insulin (by Abel's method) which we gratefully acknowledge to Dr. Oskar Wintersteiner, was also used. This material assayed 25 units per milligram. Neither of these preparations gave results any different from the action of the ordinary insulin on dogs. To show that with

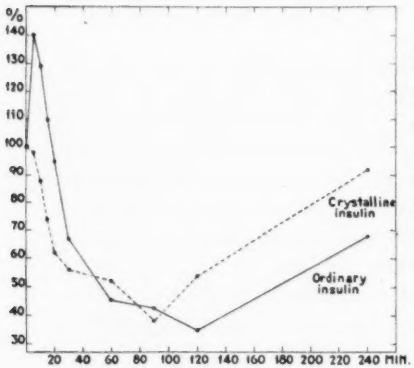


Fig. 3

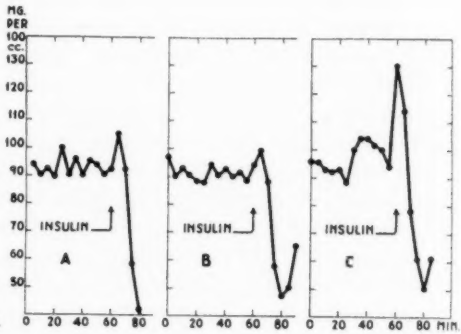


Fig. 4

Fig. 3. Effect of ordinary and crystalline insulin in cats. Dose, 1 unit per kilogram. Blood sugar values are expressed as per cent of the initial value.

Fig. 4. These three blood sugar curves have been selected to show the results when insulin happened to be injected near the beginning (A), about the middle (B), and at the top (C) of an upward oscillation. (For further discussion see text.) They also illustrate the behavior of the oscillations. The usual thing is represented in curves A and B. The oscillations will vary in height but in general conform to the relatively regular pattern shown. This pattern is found in normal as well as demedullated dogs. The cause of the irregularity is not known.

present day clinical insulin the dog is not subject to measurable initial hyperglycemia and to illustrate the semblance of slight hyperglycemia if the oscillations of the resting blood sugar are disregarded we give three curves in figure 4. The dose used was 2 units per kilogram. Curve A shows the injection of insulin near the beginning of an upward oscillation simulating an initial hyperglycemia of 13 per cent. In curve B the insulin happens to be injected in the middle of an upward oscillation, giving the appearance of 5 per cent hyperglycemia. Curve C shows the condition where at the end of an unusually large upward oscillation the insulin leads to a direct precipitate fall. Smaller doses do not lessen this semblance of

initial hyperglycemia. This is of interest when compared with the data on cats given below.

Observations on cats with 0.1 unit, 0.5 unit, 1 unit, and 2 units per kilogram intravenously. In the cats the saphenous vein was used for injection and bleeding. Using large, quiet dispositioned animals and the most considerate treatment little trouble is experienced with emotional hyperglycemia. In the few instances where struggling or other excitement occurred the result was evident and the experiments were discarded. The great similarity at all but hypoglycemic emergency levels of the curves for normal and medullectomized cats precludes any marked effect of excitement acting via adrenal medulla (8).

One-tenth unit per kilogram. Five cats. No initial hyperglycemia; minimum values at 35 to 40 minutes. The drop is not below 83 per cent of the initial level, and 75 to 90 minutes are required for return.

Five-tenth unit per kilogram. Ten cats. Initial hyperglycemia occurs (28 to 34 mgm. per cent above original value). Minimum at 35 to 60 minutes with a reduction to an average of 58 per cent. Final return to initial value occurred in 90 to 180 minutes.

One unit per kilogram. Fourteen cats. The initial rise becomes considerable (27 to 58 mgm. per cent). Minimum at 60 to 120 minutes reaching an average of 50 per cent of the original value. Return to initial value 180 to 300 minutes.

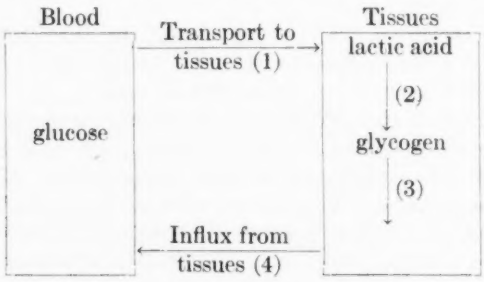
Two units per kilogram. Seven cats. Initial rise 31 to 78 mgm. per cent. Minimum at 90 to 180 minutes with about 48 per cent of original value. Return to normal occurred in 300 to 360 minutes.

Curves made up of mean values are more significant in cats than in dogs. Curves for the 36 experiments here described are given in figure 2 of the following paper (8) as controls on medullectomized cats. The results may be summarized as follows: With all except the 0.1 unit per kilogram dose there is an initial rise in blood sugar roughly proportional to the dose. The curve drops to a single minimum (no intermediate maximum) and then returns to normal or slightly above. As far as the present data on cats indicate, both intensity and duration of action may be related to the dose. (cf. Scott and Dotti, 13, on subcutaneous insulin in rabbits.)

Having noted that no initial hyperglycemia was present in dogs with ordinary insulin for clinical use, the findings in cats deserve attention, especially since the purified dry sample of nearly the same potency as crystalline insulin gave the same results. With crystalline insulin in confirmation of others the initial hyperglycemic trend was absent (fig. 3). In the cat 0.2 unit per kilogram of the non-crystalline dry Lilly preparation or about one gamma of the impurity per kilogram produces a measurable effect: (0.2 u. = 1/125 mgm. per kilo; impurity (10 per cent) = 1/1250 mgm. per kilo). Without the concomitant blood sugar lowering effect of insulin the

results would be more marked. The potency is to be adjudged still higher if not all of the 10 per cent impurity is active material. It thus belongs in the class of substances of high physiological activity (see Clark, 14, p. 21). Every individual experiment with cats showed a rise and the results were rather uniform for a given dose. Dotti (15) finds the hyperglycemic action of insulin in rabbits variable. Interest attaches to his statement that those individuals who show a marked initial hyperglycemia are more frequently protected against convulsions than those not showing it. How much of this impurity was present in various earlier preparations of insulin has been discussed particularly in the foreign literature (review by Hennequin, 16). At present the insulin for clinical use shows apparently no constant or measurable hyperglycemic effects in humans or dogs. Burger's (17) results as far as species effects are concerned seem to be somewhat at variance with those here reported.

Discussion. A working hypothesis, in agreement with most of the available data and subject to further elaboration may be stated. The basis of all considerations of blood sugar level is the cycle:



Insulin accelerates the transport and inhibits the influx of blood sugar. For literature on glycogenolysis inhibiting action of insulin see v. Ledebur (18). In this connection it might be well to avoid the term "glycogenolysis" which in common usage includes (3) and (4). Its use infers that the rate of the process is the rate of the glycogenolysing enzyme action. What Claude Bernard called the secretion of sugar by the liver, i.e., the transfer may be a separate and independently variable factor.

Comparing the first part of the blood sugar curves after intravenous injection in the dog and in the cat we see that in the dog either the transport is more accelerated or the influx is more completely suppressed. The latter is the more probable since the same proportion of the blood sugar raising impurity present in the insulin produces a marked hyperglycemia in the cat while it has no perceptible effect in the dog. In the dog the insulin apparently shuts down the release of carbohydrate from the liver to the blood more completely.

In the cat if there is only partial inhibition of influx the course of the blood sugar curve after insulin up to the time of the minimum represents a neat balance between influx and transport to the tissues. Both the time of minimum and extent of lowering are dependent on the dose. In the dog an oscillation rhythm of about 20 minutes seems to persist, the intermediate rise being an accentuated upward oscillation very noticeable on account of the previous 20 minute shut-down on carbohydrate release from the liver. Undoubtedly many factors enter the picture besides the primary action of insulin (transporting blood sugar to the muscle) and the controlled extent to which the liver allows sugar influx into the blood. The course of blood sugar plotted against time will be changed whenever the ratio between influx and transport changes. With resting blood sugar or during marked change, something resembling oscillation must occur unless from moment to moment this ratio is kept constant.

With subcutaneous insulin (details to be published later) the inhibition of influx is only partial resulting in very little difference from intravenous effects in cats but markedly modifying the curve in the dog.

In view of what has been said regarding influx and outflow of blood sugar the return of blood sugar to normal after the action of insulin deserves attention. As the inhibitory action of insulin on the liver glycogenolysis wears off the restoration of the blood sugar level follows automatically, particularly since a significant amount of carbohydrate must have reached the liver as lactic acid via the muscle. Questions relating to the rôle of the adrenal in blood sugar restorations are taken up in the following paper.

CONCLUSIONS

1. The typical response of the dog and the cat to intravenous insulin is described. In the dog the size of the dose reflects itself nearly exclusively in duration of action. In the cat both duration and intensity of action vary with the dose. In the dog marked oscillations of blood sugar after insulin occur. In the cat oscillations are not in evidence.

2. Insulin sufficiently purified to show no initial hyperglycemia in dogs may still contain enough impurity to give an initial blood sugar rise in cats. About one gamma of impurity per kilogram will produce measurable effects.

3. The significance of "glycogenolysis inhibiting action" in the two species is discussed.

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THE RÔLE OF THE ADRENAL GLAND IN BLOOD SUGAR RECOVERY AFTER INSULIN HYPOGLYCEMIA[1]

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Received for publication January 18, 1937

In the course of intravenous insulin action as reported previously (1) the blood sugar recovery phase presents a number of features which are of interest. The emergency action of the sympathico-adrenal system *at hypoglycemic levels* has been established by Cannon (2) by means of animals in whom this system has been inactivated in various ways. Few studies have dealt with blood sugar restitution when the hypoglycemia is not fatal. The justified desire to save valuable animals has led to the practice of administering glucose when symptoms appear, thus precluding observations on the frequency of recovery without restoratives (3, 4).

Our observations deal with insulin response: *A*, after medullectomy (survival experiments); *B*, after bilateral adrenalectomy (acute experiments); and *C*, after bilateral adrenalectomy in survival experiments with cortical extract treatment. Dogs and cats were used for *A*; cats for *B*; and dogs for *C*.

A. Bilateral medullectomy. The medullectomy was performed by the method first described by Houssay (5) for dogs and Zwemer (6) for cats. The operation consisted of exposing the medulla by longitudinal section through the gland and removing it by gentle scraping with a curette. For reasons given below great care was taken to avoid undue injury to the underlying cortex. In this respect the procedure differed from that employed by Houssay and by Zwemer who removed part of the cortex in order to insure complete removal of the medulla; also splanchnic nerves were not divided as in Houssay's method. In procedures of this kind the exact technique employed, the amount of injury to the tissues, the course of recovery, etc., are not without effect on the subsequent physiological reactions. Reproducible results cannot be expected unless the animals are in prime condition.

Wounds sometimes became infected, due, probably, to the local ischemic

[1] This work has been made possible by a grant in aid from Eli Lilly & Co. We also gratefully acknowledge liberal supplies of insulin and amytal.

effects of epinephrin liberated during removal of the medulla. Careful drainage and ample time for recovery may still yield a good experimental animal. Irrigation with copious amounts of salt solution at the time of operation diminishes the number of infections. After successful medullectomy appetite, activity and weight were normal.

When animals finally came to autopsy a careful search for accessory adrenals was made and none were found, except in one cat. This tissue, as others have found, was identified in section as wholly cortical.

Careful histological examination of serial sections of the operated glands revealed complete absence of medulla which was replaced by scar tissue, except in 2 cats in which minute amounts of chromaffin tissue were left. Rough calculation from serial sections indicate that the chromaffin tissue left was certainly not more than one one-hundredth of the medulla volume. Fixation in Muller formol solution (Kohn) (7) was used for the identification of chromaffin cells. The cortex showed connective tissue infiltration always involving the inner reticular layer but in some cases extending into the adjacent fascicular layer.

Blood samples were obtained from the jugular vein in dogs and from the saphenous vein in cats at the time intervals indicated in the charts. The initial blood sugar values recorded (p. 541) indicate that the cats were not unduly excited. Comparing the values with some of the older studies it must be remembered that present methods give lower values for "sugar." Sugar determinations were made according to the Shaffer-Hartman method on tungstate filtrates. In figures 1, 2 and 4 the results are expressed as per cent of the initial value. In figure 3 the values are given as milligrams per 100 cc. Animals were without food for eighteen hours. Insulin experiments were begun three to nine days after bilateral medullectomy had been completed in two stages. Throughout, the insulin used was the ordinary Lilly insulin for clinical use.

In all animals, the blood sugar response to various doses of insulin was determined before and after medullectomy. In addition control observations with all doses of insulin were made in another series of normal dogs. Using 4 medullectomized dogs the following number of experiments were carried out with varying doses per kilogram: 12 with 0.1 unit; 8 with 0.5 unit, and 13 with 1.0 unit. In the case of medullectomized cats (5 animals) the number of experiments were: 5 with 0.1 unit; 6 with 0.5 unit; 5 with 1.0 unit; and 5 with 2.0 units. Suitable rest periods were given between experiments; with the larger doses it seemed safer to wait at least 10 days between experiments.

Initial blood sugar values were not significantly changed after medullectomy. (Table 1.)

It is apparent (figs. 1 and 2) that with doses large enough to produce marked hypoglycemia and several convulsions in medullectomized animals

there was no difficulty in obtaining good spontaneous recovery of blood sugar. The blood sugar returned to the initial value within about the same time as in normal animals. Results of this kind were obtained early or late after medullectomy (earliest: 3 days; latest: 108 days).

Again we note that all the animals reported here were large, robust and healthy specimens. Recent loss of weight, wound infection, or even mild respiratory infections may be fatal.

TABLE 1

Blood sugar in dogs before and after medullectomy

Number of determinations given in parentheses

DOG NUMBER	BEFORE MEDULLECTOMY	AFTER MEDULLECTOMY
	mgm. per 100 cc.	*mgm. per 100 cc.*
1	89 (2)	85 (9)
2	98 (6)	96 (8)
3	94 (5)	96 (3)
4	96 (5)	98 (15)

In the cats similar data were obtained.

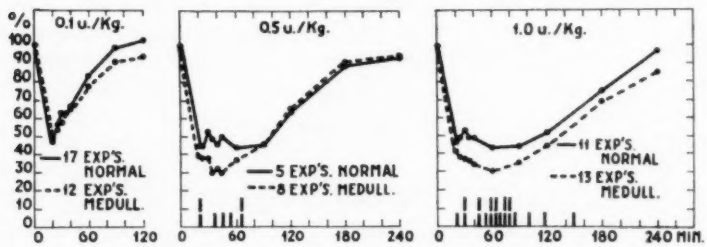

Fig. 1. The course of blood sugar in normal and medullectomized dogs after 0.1, 0.5 and 1.0 unit per kilogram of insulin. The blood sugar is expressed in per cent of the original value. In this and the following charts the black blocks along the base line stand for one convulsion in one animal at the time indicated. For total incidence of hypoglycemic symptoms see table 2.

The mean value curves for medullectomized animals indicate a more marked hypoglycemia after the medulla has been removed. Examining the individual blood sugar curves it appears that hypoglycemic symptoms are associated with blood sugar values below 40 mgm. per cent and convulsions occur when the sugar drops to very low ranges of about 30 to 20 mgm. per cent (total reducing substance).

In agreement with the emergency concepts of sympathico-adrenal action the blood sugar in normal animals as a rule just avoided the final drop to values in the critical range which in our animals appears to be about 30 to

40 mgm. per cent (total reducing substance). Dotti and Hrubetz (8) find in normal rats a narrow range where symptoms are shown but no convul-

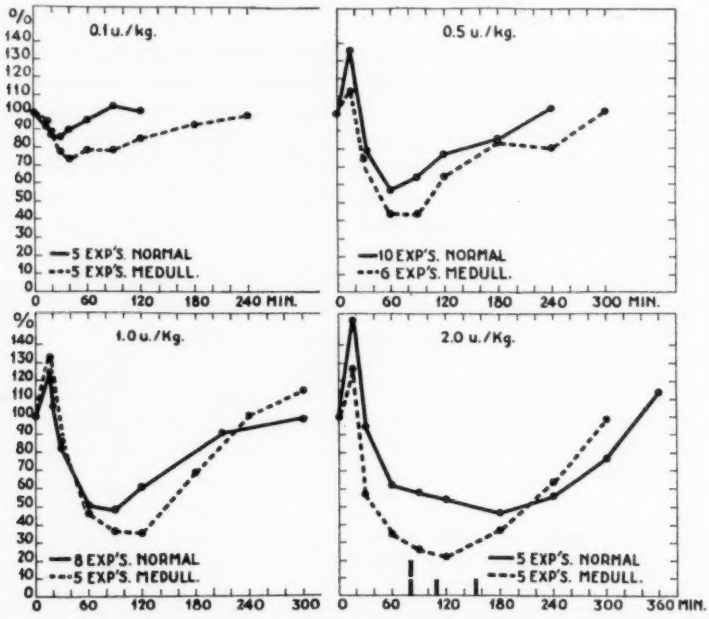

Fig. 2. The course of blood sugar in normal and medullectomized cats after 0.1, 0.5, 1.0 and 2.0 units per kilogram of insulin. Notations same as in figure 1.

TABLE 2

Incidence of symptoms

		0.1 u.	0.5 u.	1.0 u.	2.0 u.
Dogs	Normal	0/17	2/5	4/11	
	Demedullated	0/12	6/8	13/13	
Cats	Normal	0/5	0/8	1/8	1/5
	Demedullated	0/5	2/6	4/5	5/5

sions occur. Convulsions are found in rats with a mean total reducing value of 40 mgm. per cent expressed as glucose. (Cf. hypoglycemic symptoms and convulsions below.)

The blocks along the base line of the charts indicate the frequency of the convulsions and their time of occurrence in the medullectomized animals. The respective frequency of all symptoms (mild or marked) in normal and demedullated animals produced by various doses is given in table 2. The upper figure gives the number of experiments in which symptoms were shown as a fraction of the total number of experiments indicated by the lower figure.

It is apparent therefore that the striking difference in the behavior of the animal without medulla lies only in the behavior at hypoglycemic emergency levels. The blood sugar restitution is complete and not even delayed when compared with the normal. These results are in accord with Bøggild's (9) observations (1933) on insulin response of demedullated animals. Observations which he reported in an earlier paper are not in agreement with his later work.

B. Bilateral adrenalectomy. Acute and short time survival experiments in cats without cortical extract. As shown above, surgical removal of the medulla did not appreciably affect the return of blood sugar to normal values. To make doubly certain that medullary remnants were excluded and to investigate possible effects of cortex, acute experiments on bilateral complete adrenalectomy were resorted to next.

Under nembutal anesthesia both adrenals were removed and insulin experiments were performed at various intervals. In two cats 0.5 unit per kilogram was given 30 minutes after the operation. Blood sugar returned and the animals survived. After the same time interval 1.5 units per kilogram in two animals and 2.0 units per kilogram in one animal also led to restoration of normal sugar and survival. When 18 hours had elapsed since adrenal removal and 1.0 unit or 1.5 units per kilogram were given, 3 out of 4 animals succumbed in marked hypoglycemia with no tendency to blood sugar recovery. The fourth cat survived with restitution of normal blood sugar.

When 72 hours had elapsed since adrenalectomy 0.5 unit per kilogram did not allow blood sugar recovery. In two other animals with the small dose of 0.1 unit per kilogram one survived and one died in hypoglycemia.

Another detail must be given some consideration, when the time interval after adrenalectomy is short (30 minute experiments). The nembutal anesthesia and handling of the adrenals causes a moderate rise in blood sugar. The same factors leading to high blood sugar in the beginning of the experiment might also be expected to influence recovery. Two experiments were therefore performed after the hyperglycemia had worn off as illustrated in figure 3. After the initial rise, the blood sugar was followed for three hours and only then was the insulin given (1.5 and 1.65 units per kilogram). A temporary increase (see previous paper) was followed by a very marked drop reaching a level of 20 mgm. per cent in 120

minutes. From here on a gradual rise set in and six hours after the insulin injection the sugar had again attained the initial value. Experiments with normal animals under nembutal anesthesia showed that blood sugar recovery was somewhat delayed, compared to the results obtained in unanesthetized cats. The results thirty minutes and three hours after adrenalectomy are about the same in allowing blood sugar recovery to take place with relatively large doses of insulin in all animals. Eighteen hours and later much smaller doses lead to fatal hypoglycemia in the majority of cases. A summary of the thirteen experiments is given in table 3. The foregoing results in acute or short survival experiments demonstrate that recovery from hypoglycemia after insulin occurs in the absence of the adrenal glands. A reflex mechanism acting through the adrenals cannot be concerned in this response. In these operations one can be certain of complete removal of the medulla in case any doubts existed with regard to the technique of medullectomy. The difference between medullectomy

TABLE 3

NUMBER OF EXPERIMENTS	TIME AFTER ADRENALECTOMY	INSULIN U/KGM.	BLOOD SUGAR RECOVERY	SURVIVAL
5	30 minutes to 3 hours	0.5–2.0	Normal	5 Survived
4	18 hours	1.0–1.5	1 Normal 3 No recovery	1 Survived 3 Died
2	72 hours	0.5	No recovery	2 Died
2	72 hours	0.1	1 Normal 1 No recovery	1 Survived 1 Died

and complete adrenalectomy after the longer time intervals is either related to the magnitude of glycogen stores dependent on cortical activity (see Britton, 10) or an unknown cortical factor concerned with blood sugar regulation which persists for several hours after removal of the adrenals. Further evidence that the phenomenon is related to the cortex follows.

C. Bilateral adrenalectomy in dogs. Survival experiments with cortical extract treatment. Bilateral adrenalectomy was performed in three male dogs weighing 11.2, 12.3, and 14.3 kgm. respectively. The glands were removed by the lumbar route in two stages, intervals of 13 to 27 days elapsing between operations. One of the animals had been used previously for demedullation studies. The dogs were kept on cortical extract (Parke Davis & Co.)[2] rated at 40 dog units per cubic centimeter. To assure sufficiency at least 3 units per kilogram per day were given for maintenance with still higher doses immediately after operation. Insulin experiments were performed within nine to fourteen days after the final stage of adre-

[2] Grateful acknowledgment is made to Dr. Oliver Kamm of Parke Davis & Co and to Dr. J. J. Pfiffner of the Biochemistry Department of Columbia University.

nalectomy, in animals normal with regard to appetite and activity. In two dogs no lasting change in weight occurred, and in one there was a slight loss. The non-protein nitrogen levels after the removal of the second adrenal under cortical extract treatment were at times possibly a little above usual normal values.

Dog 1.....	44	42	43	42	35	mgm. per cent NPN
Dog 2.....	33	21	25	35	31	mgm. per cent NPN
Dog 3.....	53	49	49	38	35	36 mgm. per cent NPN

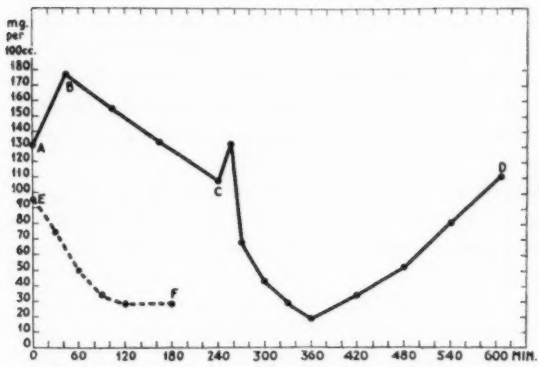

Fig. 3

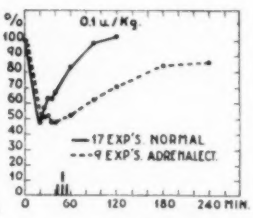

Fig. 4

Fig. 3. Insulin effects at 3 hours (A, B, C, D) and 72 hours (E, F) after adrenalectomy. Blood sugar is expressed as milligrams per 100 cc. At *A* nembutal anesthesia was begun. During the time *A* to *B* double adrenalectomy was carried out. Three hours (*B* and *C*) were required for the return of blood sugar to the normal range. At *C* insulin was given (1.5 unit per kilogram). At *D* the blood sugar had returned to normal and the animal was out of the anesthesia. In the interval the blood sugar had fallen to a level of 20 mgm. per cent without convulsions (anesthesia). *E* to *F* shows in another cat the result of 0.5 unit per kilogram of insulin 72 hours after adrenalectomy. Death at *F*.

Fig. 4. Course of blood sugar after insulin (0.1 unit per kgm.) in normal and adrenalectomized dogs under treatment with cortical extract. Notations same as in figures 1 and 2. Nine experiments resulted in 4 convulsions. All animals however showed hypoglycemic symptoms with this dose. Compare table 2 for symptoms in demedullated dogs.

Blood sugar values before and after adrenalectomy are given below. The values before adrenalectomy were obtained (each on a different day) within two weeks before operation. Those after adrenalectomy were spread out over the remaining life of the animals. The dogs were sacrificed after 29, 34 and 44 days respectively and careful search at autopsy revealed absence of all adrenal tissue. One dog, which was not used in the insulin experiments, is included.

Observations on the response to insulin were made with 0.1 unit per kilo-

gram. Three experiments were performed upon each animal. The daily requirement of cortical extract was given about one hour before the injection of insulin. With 0.1 unit of insulin per kilogram symptoms were not encountered in normal animals. After complete adrenalectomy this dose produced weakness, unsteadiness of gait and twitching in all of the animals and convulsions appeared in four out of nine of the experiments. After each experiment glucose and an extra dose of cortical extract were given and seven to fifteen days allowed to elapse before the next experiment. As is well known these animals are very sensitive and great care must be exercised to make certain that they are in good condition at the time of experiments.

The blood sugar response was characterized by a prolonged hypoglycemia and marked retardation in recovery. In eight experiments restoration to initial blood sugar levels was not attained until three hours or

TABLE 4

Blood sugar values in milligrams per 100 cc.

Dog 1:										
Normal	93	110	98	95	96	98				
Adrenalectomized	94	84	89	91	88	88	83			
Dog 2:										
Normal	83	90								
Adrenalectomized	88	93	84	91	94	82	91	91		
Dog 3:										
Normal										
Adrenalectomized	83	90	91	91	88	95	96			
Dog 4:										
Normal	86	74	85	88	89	86	73	87	82	89
Adrenalectomized	89	84	80	76	81	73	71	77		

longer after the injection of insulin; in one experiment full return took place in two hours. Figure 4 shows the comparative results obtained in normal and adrenalectomized animals, each curve representing mean values (17 normal; 9 adrenalectomized).

These results demonstrate that in survival experiments following adrenalectomy blood sugar restitution after insulin is markedly delayed and the tendency to develop hypoglycemic symptoms is greatly increased. Under treatment with cortical extract the totally adrenalectomized dogs give hypoglycemic symptoms with smaller doses than the medullectomized animals. Compared with the acute adrenalectomy of more than a few hours standing cortical extract seems to improve blood sugar restitution. With the amounts given of the particular extract here used the rate of blood sugar return was much delayed however when compared with normal or demedullated animals.

In dogs and cats we have verified the fact first pointed out by Porges

(11) in 1910 that glycogen diminishes rapidly (low after a few hours) when the adrenals are removed (see also Britton, 10). The three cortin treated dogs mentioned above, however, had 2.3 per cent, 2.2 per cent and 2.2 per cent of glycogen in the liver.

Discussion. One finds frequent references in the literature to epinephrin secretion consequent to insulin injection as taking a part in the restitution of blood sugar to normal. Cannon stated expressly that the epinephrin secretion is only a part of the sympathico-adrenal mechanism and also pointed out that as the blood sugar rises beyond emergency levels epinephrin secretion as indicated by the denervated heart is rapidly minimized. The return of blood sugar to normal had no bearing on Cannon's immediate problem and he recorded no full recovery data. In following the blood sugar through convulsions, we have been able to show that absence of the adrenal medulla has no effect on the occurrence of restitution or the time occupied by it. It appears therefore (in agreement with Cannon) that the phenomenon related to blood sugar, described as emergency action, confines itself entirely to the neighborhood of the "critical level." The rest of the blood sugar return is to be accounted for by other mechanisms. The results recorded here on removal of the cortex, however, indicate the possibility that this organ if disturbed may distinctly enter the picture of blood sugar regulation and other carbohydrate changes. Its relation to glycogen storage has been known for a long time. The fact that even with 2 per cent glycogen in the liver the blood sugar restitution in cortin treated adrenalectomized dogs is very significantly delayed still further stresses the rôle of the cortex and adds to the indications in the literature of cortex effects amounting to more than mere ability to promote glycogen storage. The fact that adrenalectomized animals even with liberal cortin treatment, normal NPN values and fair carbohydrate reserves, show symptoms with smaller doses of insulin than either medullectomized or normal animals stresses the cortex effects on muscle maintenance and the uncertainty regarding particular cortex preparations and particular doses as complete replacement therapy. The increased sensibility to insulin after adrenalectomy cannot be attributed entirely to the absence of the medulla as suggested by Harrop (12).

Our original objective in these experiments was to ascertain to what extent changes in adrenal function consequent to removal of the pancreas might be held accountable for differences in insulin response between normal and depancreatized animals. We have elsewhere presented (13) data which showed that injected epinephrin has little or no effect on the blood sugar during insulin action and the question was raised whether the same is true of epinephrin secreted in the body.

We now see no cogent reason for assuming any relation of the adrenal medulla to the diabetic insulin response but believe that the rôle of the cortex merits further work in this direction.

CONCLUSIONS

1. The adrenal medulla is not essential to blood sugar recovery after insulin hypoglycemia.

2. Bilateral removal of the medulla without further interference with normal function abolishes the "sympathico-adrenal emergency mechanism" at hypoglycemic levels.

3. Absence of both whole adrenal glands does not prevent recovery from hypoglycemia but may delay it. Acute experiments demonstrate this as well as survival experiments with animals under cortical extract treatment.

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A STUDY OF AFTER-CONTRACTION

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Received for publication January 18, 1937

The phenomenon termed after-contraction is shown in the following manner: when any set of muscles, such as those involved in raising the extended arm or leg, is voluntarily kept in action for a period of time, and then voluntarily relaxed, there occurs involuntarily a repetition of the original movement, accompanied by a sensation of lightness or floating upward of the part. This phenomenon has long been known. It was described by Salmon at a meeting of the Italian Neurological Society in 1914, and again independently by Kohnstamm (1) in 1915, under the heading Nachbewegung (Katatonus Versuch). Kohnstamm likened it to catalepsy, and gave as an explanation that when a set of muscles is extended, and in a state of tension, the muscles take on a new equilibrium posture to which they return as soon as freedom of movement is brought about by the relaxation. He assumes that a special tonus innervation of the muscles is responsible. Rothmann (2), Csiky (3), Salomonson (4), Pinkhoff (5) and Matthaei (6) have also reported on the after-contraction and have given various explanations of its mechanism. Rothmann believed that the reaction depends on a continuation of subcortical impulses after cessation of the voluntary movement, the stimulus coming from the cerebellum. Csiky compares the reaction to after image and explains it as an idiomuscular contraction. Matthaei agrees with Rothmann in ascribing the cause to a duration of excitation of subcortical centers. He found that cocainizing the sensory nerves brought about a disappearance of the subjective symptoms but no alternation of the after-contraction. Pinkhoff made a rather extensive study of the reaction. He finds that it varies in different individuals and in different muscle groups, that it may be lessened by fatigue, and that it is accompanied by an action current in the peripheral nerves. He concludes that the spinal motor cells remain in a state of excitability after the primary stimulation, partly from the voluntary impulses causing the initial movement and partly from proprioceptive impulses arising in the muscles.

In a second communication Salmon (7), on the basis of his studies on normal subjects and on those with cord or brain lesions, rejects the possibility of a peripheral or spinal orgin of after-contraction. He assumes a

cortical origin, which he defines as a kinesthetic image, which persists in the motor cortex after cessation of the various stimuli involved in the original contraction. He likens it to the hallucinatory after-images produced by an intense sound or a bright light.

After-discharge, facilitation, fatigue and other factors connected with reflex activity and with direct stimulation of cortical areas have been studied by many workers. In such studies, animals which have been decorticated, decerebrated, or are under the influence of anesthetics have been employed, consciousness or voluntary movements being abolished. The after-contraction here described has been studied in a normal animal, man, and while it does not allow of the analysis possible in experimental lower animals, it presents a composite picture lacking in these. It is of especial interest to us in that it allows a study of the effects of selective drug action and of cerebral and spinal pathological conditions in modifying it.

EXPERIMENTAL PROCEDURE. In obtaining a record of the after-contraction, flexion at the hip was employed because the reaction is more marked than in the other muscle groups and, in addition, is easily recorded and studied. The following procedure is carried out. The subject stands on a platform, supporting his weight on one leg, the other hanging free, with balance maintained by the arm on the supporting leg side resting lightly on the arm of a chair. To an ordinary riding spur fastened to the heel of the free hanging leg is attached a cord passing over two pulleys to give proper direction and ending at a lever, arranged to record the leg movement on a kymograph. Also attached to the spur is a second cord passing over a pulley, and connected at its other end with a receptacle in which various weights can be placed. This cord is attached to the spur by means of a spring catch and can be disconnected with no delay.

At a word of command the leg is lifted, and held elevated for a given time. At a second command the leg is relaxed and the weight cord disconnected. The after-contraction occurs promptly and is registered on the kymograph. In some of the experiments the weight cord was not used, the weight lifted being that of the leg alone. This, we estimate, corresponds roughly to about $\frac{1}{6}$ of the body weight in a person of average proportions. A kymograph record of the after-contraction is shown in figure 1. The plateau, *A*, in the beginning, records the voluntary movement of the leg in lifting and holding up the weight. The drop, *B*, at the end of the plateau, records the relaxation of the leg, at which point the weight is detached. The after-contraction record, *C*, follows, showing a steady ascent, a sharp peak and a steady fall.

The mechanism which gives rise to the after-contraction is much more complicated than in the case of the after-discharge seen in animal studies on reflex activity or cortical stimulation. The stimulus originates with

FIG. 1

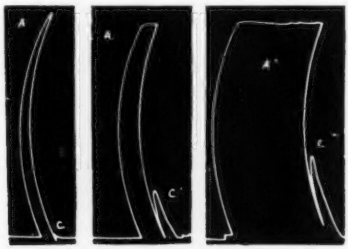

FIG. 2

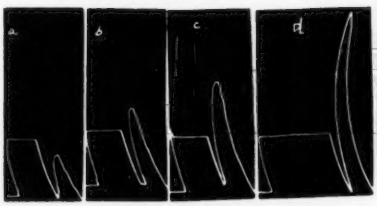

FIG. 3

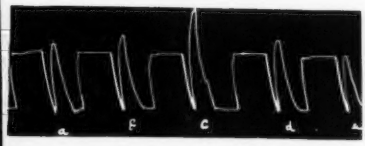

FIG. 4

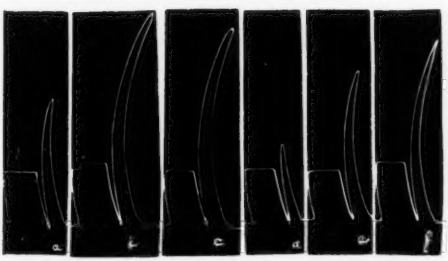

FIG. 5

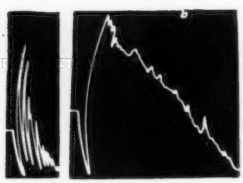

FIG. 6

Fig. 1. Typical record. *a*, voluntary elevation of leg; *b*, voluntary relaxation; *c*, involuntary after-contraction. Time in seconds.

Fig. 2. Effect of prolonging stimulus. The preliminary contractions *A*, *A'*, *A''*, record the voluntary elevation of the leg with no weight attached. The after-contraction, represented by *C*, *C'*, *C''* respectively, is absent in the first record, increasing in the second and highest in the third.

Fig 3. Effect of load and duration. *a*, load of 1 kilo—10 seconds; *b*, load of 3 kilos—10 seconds; *c*, load of 5 kilos—10 seconds; *d*, load of 3 kilos—20 seconds.

Fig. 4. Facilitation and fatigue. Series of after-contractions obtained consecutively with no delay between cycles. Load of 3 kilos. Duration 10 seconds. *a*, *b*, *c*—facilitation; *d*, *e*—fatigue.

Fig. 5. Reinforcement. *a*, normal (3 kilos—10 seconds), left leg; *b*, with dorsiflexion of left foot; *c*, with left arm lifting 2 kilos; *d*, with right arm lifting 2 kilos; *e*, with both arms each lifting 2 kilos; *f*, with Jendrassik procedure.

Fig. 6. Normal variations. *a*, oscillatory type of after-contraction; load 6 kilos—15 seconds; *b*, prolonged clonic type of after-contraction; load 3 kilos—15 seconds. All figures reduced one-half from originals. The time record in figure 1 applies to all the records. In the records taken a height of 1 cm. corresponds roughly to a rotation of the leg through 10 degrees.

the command to raise the leg, and travelling along the acoustic nerve sets in action the coördinating mechanism necessary for voluntarily raising the leg and holding it raised. At the second command the extremity is relaxed and it seems probable that an actual but short inhibition occurs before the after-contraction begins. Also in elevating the extremity and maintaining the elevation, especially when a heavy weight is lifted, some effort to preserve postural balance is required, and some tension is put on the supporting extremity.

EXPERIMENTAL DATA. *Duration of stimulus.* When the extremity, the leg extended and no weight attached, is raised and immediately relaxed, usually no after-contraction occurs. If the contraction period is prolonged, the after-contraction appears, its extent increasing with increase in the duration of elevation. An elevation lasting one second may bring on the after-contraction, one lasting about 25 seconds the maximum effect. See figure 2.

Strength of stimulus. When the duration of stimulus, i.e., the period of the elevation of the extremity, is kept constant and the weight lifted increased, the after-contraction also increases. The maximum is reached when the weight is the heaviest that can be lifted. The increase in amplitude of the after-contraction does not vary linearly with increase in load but saturates fairly rapidly. See figure 3, *a*, *b*, *c*.

Combination of increase of duration and of strength of stimulus. This increases the after-contraction to a greater extent than when either of the factors operates singly. See figure 3 *d*.

Facilitation and reinforcement. When the extremity, with no load attached, is raised and immediately relaxed, no after-contraction occurs, as has been stated. If however this cycle is repeated with no delay, the after-contraction may appear after as few as three elevations. As the number of elevations is increased, the greater the after-contraction becomes. With a fixed load attached, and the elevation maintained for a fixed period, with frequent repetition of elevation an increase in after-contraction occurs which is greater than when no load is attached, provided that the element of fatigue is not interfering. See figure 4 *a*, *b*, *c*.

When during the period of elevation of the extremity, the foot is strongly dorsiflexed, the after-contraction is markedly increased. If instead of flexing the foot, the ipsilateral arm is abducted at right angles to the body, a weight of 2 kilos being held in the hand, there also occurs an increase in the after-contraction. If however instead of the ipsilateral arm, the contralateral one is raised, the after-contraction is usually lessened, although it may be increased. Raising both arms results in an after-contraction which is intermediate in relation to that seen after raising the two arms singly. See figure 5.

The Jendrassik method (pulling the arms apart against resistance from

the interlocked fingers), is commonly used to reinforce tendon reflexes. Its effects on after-contractions are variable. In some individuals there is an increase, in others a decrease. Both ipsilateral and contralateral impulses affecting arm areas are involved here, and as might be expected the results correspond to those described in the preceding paragraph. In short, voluntary ipsilateral muscle contractions accompanying the cycle increase the after-contraction. Contralateral contractions cause variable effects, in some instances an increase, in others a decrease.

Inhibition. With a suitable duration of elevation of the extremity and load lifted, a large after-contraction is seen. If at the moment of relaxation a voluntary and momentary tension of the extremity in the opposite direction is made, there may be no after-contraction. If without consciousness on the part of the subject, a slight restraint of the upward movement after relaxation is made, the after-contraction is delayed. The mere knowledge on the part of the subject that restraint may be made can prevent the after-contraction.

Fatigue. When the cycle of after-contraction is repeated at short intervals the after-contraction eventually progressively decreases and finally is abolished. The time and degree of decrease depends on the weight lifted and the duration of elevation of the extremity. With a weight of 6 kilos and a duration of 10 seconds and a repetition of the cycle at 3 seconds' interval, the decrease may be seen as early as the third repetition and can be abolished with the fifth. With long rest intervals, and less weight and duration, it may be an hour before fatigue appears. The subjective sensation of fatigue appears before the after-contraction lessens. It is felt especially in the supporting leg. See figure 4 *d*, *e*.

Latent period. It is impossible to measure accurately a latent period with the procedure used here. The relaxation of the extremity is a conscious one, and seems to include not only a cessation of the stimulative impulse but also some direct inhibition. In general, however, the after-contraction begins in about $\frac{1}{5}$ of a second after relaxation has been made. With fatigue its onset may be delayed as long as 3 seconds.

Individual variations. While the effects on the after-contraction just described are those which may be demonstrated on most subjects, individual variations are quite common. The record of the after-contraction usually shows a steady rise, a fairly sharp peak and a steady fall, of about the same duration as the rise, the average total duration being about 10 seconds. In studying a large number of subjects, large variations in amplitude were observed, ranging from no rise to one limited only by the maximum range of elevation anatomically possible. The duration of the after-contraction in some instances was as short as 3 seconds, in others as long as 40 seconds. In contrast to the tonic contraction there may be the "epileptoid" or clonic after-contraction in which the rise is not steady but

jerky, the peak may be prolonged and also uneven, and the fall may be prolonged and uneven. In some instances, an oscillatory type of after-contraction has been observed. See figure 6. Despite these individual variations, each subject shows a marked constancy in the form of the curve over long periods of time. A state of general fatigue in a subject affects the after-contraction responses, in some instances preventing its occurrence. This state is perhaps responsible for variations in the after-contraction seen in some individuals on different days and at different times of day. In these instances one possible factor is the varying degrees of tension and difficulty in relaxations seen in general fatigue. Fatigue with repetition of cycles occurs more promptly in some subjects than in others. In those individuals showing no after-contraction or a very slight one, facilitation, reinforcement or practice may evoke the response, and drugs such as caffein and alcohol may also bring out the after-contraction. It is probable that an unconscious inhibition prolonged beyond the relaxation period accounts for the absent after-contraction.

Discussion. The characteristics of after-contraction, as we have described them, correspond to those of after-discharge. As has been stated, numerous accounts of after-discharge are found in the literature. It is a property of neurones, motor or sensory, which manifests itself in continued activity after cessation of the original stimulus. In the case of motor neurones this results in a continuation or renewal of muscle contraction with an accompanying action current in the motor nerve. Muscle itself does not possess this property, since no after-discharge follows direct stimulation of its motor nerve (8). The after-discharge is accepted as an enduring central excitatory state, for which such causes as physical rearrangements, chemical substances and delayed afferent impulses have been suggested (9).

After-discharges emanate from many parts of the central motor apparatus. They are observed in the reflexes of the spinal animal and are more marked in the reflex reactions of the decerebrate animal (10). They are not found in all reflex phenomena, being small in flexor and absent in stretch reflexes, whereas they are very marked in crossed extensor reflexes (11). Proprioceptive impulses modify the after-discharges considerably but are not necessary for their production (12). The most striking after-discharges emanate from the cortex following direct stimulation. Graham Brown and Sherrington (13) have described both a tonic and an "epileptoid" after-discharge originating from the motor area of the cortex. Bucy (14) has shown that epileptiform after-discharges are more readily obtained from the premotor area.

The after-discharge is amplified by an increase in the intensity of the original stimulus, and by an increase in its duration (15). It can be easily cut short by inhibition, (16) but may reappear when the inhibition

is removed. These factors affect after-contraction in a similar fashion. Facilitation, reinforcement and "fatigue" are easily demonstrated in reflex phenomena. In after-contractions their effects are equally well shown.

In the after-contraction which we have described, there seems no doubt that the central link in the chain of events is a persistent excitatory state in the cortical motor neurones. The involuntary movement is in fact a replica of the coördinated volitional movement which is necessary for its production. Like voluntary movements it brings into association widely scattered areas of the nervous system, each capable of exercising an influence in the final result. The localized response characteristic of the after-contraction, its duration, its responsiveness to inhibition, facilitation, reinforcement, fatigue and to drugs acting on the cerebrum, separate it from the after-discharge due to persistence of activity in the spinal neurones. On the other hand, the cortical excitatory state shown to occur in the after-discharge following direct cortical stimulation must also be present in the after-contraction following conscious stimulation.

In experiments eliciting after-discharge, spinal reflexes, decerebrated or decorticated animals, or those under the effects of narcotics have been studied. With such procedures many factors normally present in the intact animal are absent and too broad generalizations concerning facilitation, inhibition and fatigue should not be made. The selective action of bromides and caffein in modifying after-contraction demonstrates the sensitivity of the mechanism (17). The reinforcement by contraction of ipsilateral but quite separate muscle groups, the inhibition by contra muscle contraction would imply definite patterns of cortical spread, but here again instances are noted in which a contralateral stimulus increases the after-contraction. It is hardly to be expected that a mechanism which brings in such widespread participation of central nervous activity would be uniform for all individuals. But in the composite picture of the after-contraction presented by the normal intact individual it should be possible, by suitable means, to make clear the rôle of some of its component parts.

There is a suggestion concerning the importance of after-contraction we venture to make. In walking or running, there is a repetitive movement, perfectly coördinated, which eventually is made with almost no conscious effort, indeed the movement seems entirely automatic. It is easily believable that a basic factor involved here is that represented by after-contraction, a sustained, excitatory state of cortical neurones, responding in a rhythmic fashion to stimuli of which the subject is unconscious. Also the awkwardness and restraint accompanying complicated movements may be considered due to enduring excitation of different interfering cortical areas, and the smoothness of performance which comes with practice as an expression of a proper adjustment of facilitating and inhibitory factors.

SUMMARY

1. A description of after-contraction as it occurs in normal individuals is given.

2. The effects of facilitation, reinforcement, inhibition, fatigue and other factors which modify the reaction are described.

3. It is suggested that after-contraction is a cortical function entering into many of the phases of coördinated muscular movements.

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A STUDY OF WATER INTOXICATION IN THE INTACT AND ADRENALECTOMIZED DOG AND THE INFLUENCE OF ADRENAL CORTICAL HORMONE UPON FLUID AND ELECTROLYTE DISTRIBUTION

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Received for publication January 19, 1937

Investigators who have studied the problem of water intoxication agree, for the most part, that continued forcing of water eventually results in such an accumulation of fluid as to upset the salt-water balance of the body. The primary cause of the convulsions and death of the animal, in those cases terminating fatally, is apparently due to excessive hydration of tissue cells. An adequate discussion of the entire problem will be found in the following references: Rowntree, 1923, 1926; Green and Rowntree, 1924, 1927; Misawa, 1927; Underhill and Sallick, 1925; Harding and Harris, 1930; and Helwig, Schutz and Curry, 1935.

It has been clearly demonstrated that the serum sodium and chloride levels are low in the water intoxicated animal exhibiting symptoms, and the decline in the concentration of these electrolytes is much greater than can be accounted for by dilution of the blood. According to current ideas regarding electrolyte and fluid balance, this marked decrease in serum and extracellular sodium and chloride would create a hypotonic extracellular environment and result in disturbance of osmotic equilibrium between intracellular and extracellular compartments of the body with consequent shift of large volumes of water into the cells owing to higher concentration of intra- to extracellular electrolyte.

It occurred to us that if water intoxication is due to excessive hydration of cells associated with low serum sodium and chloride, the water and electrolyte disturbances are analogous to those existing in adrenal insufficiency in the adrenalectomized dog depleted of sodium and chloride by glucose injection. Cortical hormone should, therefore, prove effective in water intoxication. Some previous observations made in this laboratory lent support to the idea (Pfiffner, Swingle and Vars, 1934).

METHODS. The adrenalectomized dogs used were healthy, vigorous animals at peak weight, and had been operated for periods varying from five months to one year previous to use in the experiments, and main-

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tained in normal condition by adequate daily injections of cortical hormone. The intact animals were strong active dogs of approximately similar size and weight. During experimentation the animals were kept in metabolism cages without access to food and no water was allowed except that given by stomach sound. Distilled water was used for producing intoxication and was administered at the rate of 25 cc. per kgm. of body weight per hour until the onset of convulsions. When vomiting occurred the rate of administration was slowed. All dogs were fed their usual ration on the day previous to use in the experiments.

The urine was collected under toluene and any vomitus or regurgitated fluid was either readministered by stomach sound or else analyzed for sodium and chloride.[2] The cortical hormone used for injection was salt-free, i.e., was not even rendered isotonic by addition of sodium chloride.

Water intoxication in the intact dog with spontaneous recovery. Complete data obtained from study of a representative case of this series of four dogs are shown in table 1A. The total quantity of water required to produce intoxication varies with the individual dog. However, the amount retained which is necessary to induce convulsions does not vary widely in animals of approximately similar size and weight. The quantity of water retained, over and above that eliminated as urine or vomitus, is the important factor and not the total quantity given.

The dogs also vary greatly as to the urine volume excreted during the administration of fluid; some urinate freely thus requiring a large total intake to induce convulsions, whereas others form little urine and develop convulsions on a considerably smaller total fluid intake. Vomiting, which may be a prominent symptom in some animals, can be eliminated as a complicating factor by readministration of the vomitus along with the succeeding doses of distilled water.

The intact dog usually recovers spontaneously from water intoxication if forcing of water is discontinued at the time of the onset of convulsions. The symptoms of intoxication last for some hours, until the kidney resumes its excretory function and the urine flow is well established. Following the passage of urine, the exaggerated nervous hypersensitiveness disappears, and normal strength and vigor return when the excess body fluids are eliminated. The writers attribute the spontaneous recovery of the intact dog to the activity of the animal's own adrenal glands which apparently secrete sufficient hormone to bring about a normal redistribution of body fluids.

[2] The methods for blood and urine analysis have been repeatedly referred to in earlier papers of this series (e.g., Swingle, Parkins, Taylor and Hays, 1936a) with the exception of serum potassium, F. H. L. Taylor, J. Biol. Chem. **87**: 27, 1930; urea nitrogen, O. Folin and A. Svedberg, J. Biol. Chem. **88**: 77, 1930; urine chloride (modified Volhard-Harvey titration) J. P. Peters and D. D. Van Slyke, *Quantitative Clinical Chemistry*. Baltimore, Maryland **2**: 833, 1932.

During the period of water administration, the serum sodium and chloride decline to low levels—as low or lower than those characteristic of the adrenalectomized animal moribund from adrenal insufficiency—and return to normal levels during the recovery interval, despite the total lack of food

TABLE 1

Water intoxication

DATE	TIME	SERUM SODIUM PER LITER	SERUM CHLORIDE PER LITER	SERUM K PER LITER	HEMOGLOBIN	RED BLOOD CELL VOLUME	R. B. C.	UREA	SUGAR	PULSE	BLOOD PRESSURE	WEIGHT	DISTILLED H_2O INTAKE	URINE VOLUME	URINE SODIUM	URINE CHLORIDE	FLUID RETAINED	REMARKS
A. Normal dog—spontaneous recovery																		
	hours	*m.-eq.*	*m.-eq.*	*m.-eq.*	*gms. per cent*	*per cent*	*mil.*	*mgm. per cent*	*mgm. per cent*	*min.*	*mm. Hg*	*kgm.*	*cc.*	*cc.*	*m.-eq.*	*m.-eq.*	*cc.*	
11/16/36	8:40 a.m.	144.8	114.2	6.7	12.6	38.1	7.64	30.3	102	96	104	10.9						Normal
11/16/36	7:00 p.m.	124.0	94.4	5.7	9.3	33.0	4.32	17.1	109	108	110		3025	1400			1625	Slight weakness
11/16/36	9:30 p.m.	122.5	93.2	5.2	11.1	41.7	5.40	16.0	134	120	96		3500	1985	9.5	19.1	1515	Convulsions
11/17/36	11:35 a.m.	143.0	108.6	6 8	10.8	36.3	4.26	16.6	109	92	90	10.7	0	730	9.75	4.99		Normal
B. Adrenalectomized dog—recovery on extract																		
11/24/36	8:30 a.m.	139.3	107.2	5.1	11.4	43.7	6.04	22.2	93.5	92	108	10.8						Normal
11/24/36	2:10 p.m.	119.6	88.8		11.5	44.5	6.44						1325	0	0	0	1325	Slight weakness
11/24/36	4:30 p.m.	117.6	82.0	5.8	13.3	51.2	7.32	24.7	89.0	92	98		1475	190	18.7	17.8	1285	Convulsions extract
11/25/36	9:45 a.m.	128.8	98.8	7.2	12.5	46.2	6.58	25.3	93.5	116	96	10.4	0	785	11.2	10.7		Appears normal
C. Adrenalectomized dog—recovery at low levels of Na and Cl																		
9/ 8/36	9:00 a.m.	139.8	109.6	6.2	14.5	39.3	5.4	32.7	82	88	94	7.3						Vomited*
9/ 8/36	3:40 p.m.	111.5	80.8	9.3	14.2	44.4	6.0	46.5	71	112	86		1400	0	0	0	860	Convulsions extract
9/11/36	12:00 m.	115.9	81.4	6.4	15.5	38.6	6.6	133.3	96	116	80	6.6	110	410	3.0	1.96		Bright, active†

* Vomited total of 540 cc.—12:40 and 1:45 p.m.

† Eats full ration but still somewhat weak.

or salt intake throughout the experimental period. The serum potassium does not change significantly except that, in general, it tends to rise slightly during recovery (table 1A).

The loss of sodium and chloride in the urine during water intoxication

appears not to be sufficient to account adequately for the diminution of these electrolytes in the serum and extracellular fluids. It will be observed (table 1A) that during recovery the dogs excrete as much sodium (the chloride excretion is less) in the urine as they do during the period of water intoxication and yet in the face of this continued loss the serum sodium and chloride rises to approximately normal within fourteen hours.

Hemoconcentration is apparently a minor factor in water intoxication in the intact dog. The blood is diluted during the period of water administration, concentrates shortly before the onset of convulsions, and again shows dilution during the recovery phase following excretion of large quantities of urine. The blood urea nitrogen and arterial pressure do not change significantly. The blood sugar may rise sharply with the beginning of the convulsive seizures (table 1).

Water intoxication in the adrenalectomized dog maintained in normal health and vigor by maintenance doses of cortical hormone. This type of experimental animal differs fundamentally from the intact animal when subjected to water intoxication. The intact dog spontaneously recovers when fluid injections are stopped after the first convulsion, whereas the equally healthy and vigorous animal lacking adrenals invariably dies unless given either hypertonic salt or cortical hormone (salt-free) by vein. It has been our experience that these animals do not spontaneously recover even when the forcing of fluid is promptly stopped with the onset of the first convulsion.

Table 1B gives the essential data obtained from a representative case of this series of experiments. The serum sodium and chloride drop to low levels, but the serum potassium changes little if at all during the period of water administration. Hemoconcentration as indicated by changes in hemoglobin, erythrocyte count and red cell volume, increases significantly with the onset of definite symptoms although it is never as marked as during adrenal insufficiency.

The adrenalectomized dog requires less fluid intake to induce convulsions than does the intact animal. One reason for this is that they urinate less freely (some not at all) during the water-forcing period. The total quantity of water retained compares favorably with that amount requisite for inducing convulsions in the normal dog (compare A and B, table 1).

Prompt measures are required to save these animals once convulsions develop. Intravenous injection of salt-free cortical hormone (3 cc. per kgm.) restores these prostrate dogs to normal health and activity. Complete recovery occurs in the absence of any food or water intake. The serum sodium and chloride levels rise sharply but even with full recovery may remain below normal. The serum potassium tends to rise during the recovery interval, and attains its peak at the end of this period when the

body fluids have been effectively redistributed and the hemoconcentration is decreasing.

During recovery on extract, blood dilution and a marked diuresis occurs with outpouring of a large volume of urine containing sodium and chloride. Despite the fact the serum concentration of these ions is low, the dog recovering from water intoxication continues to excrete them in the urine.

Comparison of the quantities of sodium eliminated in adrenalectomized and intact dogs during the period of water administration reveals that the dog lacking adrenals excretes more of this ion into the urine than does the normal dog (table 1, A and B). The volume of water accompanying this loss of sodium is less in the adrenalectomized than in the intact dog.

Water intoxication in the adrenalectomized dog and recovery with low levels of serum sodium and chloride. By administering large intravenous doses of salt-free cortical hormone, it is possible to revive the adrenalectomized dog prostrate and in convulsions from water intoxication to activity and vigor with levels of serum sodium and chloride far below those characteristic of the animal dying from adrenal insufficiency. Table 1C shows the pertinent data. The blood changes were similar to those discussed in previous experiments. Extract was given intravenously when the animal developed convulsions and thereafter given daily for three days. The animal had a difficult recovery since no urine was passed for more than forty-eight hours. With the onset of diuresis, recovery was rapid, and at the termination of the experiment the animal was bright and active although still showing some leg weakness. During the protracted recovery interval, while the dog was anuric, the blood urea nitrogen rose to 133.3 mgm. per cent. The chief point of interest is that the animal recovered at low serum electrolyte levels on cortical hormone. However, the serum sodium did show some rise (table 1C).

Revival of the adrenalectomized dog from water intoxication with hypertonic saline. The adrenalectomized dog, prostrate and in convulsions from water intoxication, can be revived in dramatic fashion by intravenous injections of salt. The essential data are shown in table 2A. Following the onset of convulsions 20 cc. of a 20 per cent saline solution were injected intravenously. A few hours later the dog appeared bright and active and was urinating freely. The serum sodium and chloride returned to normal levels. Rowntree (1926) was the first to demonstrate the effectiveness of hypertonic saline solution on the normal dog prostrate from water intoxication.

The effect of a high salt diet and intravenous injections of hypertonic saline upon the development of water intoxication in the adrenalectomized dog. The animals were given large amounts of sodium chloride in their food for three days previous to use in the experiments, plus the usual maintenance dose

of cortical hormone. The salt totaled 19 grams for the three days. When the fluid retention approximated 1400 cc. the animals developed slight

TABLE 2

Water intoxication

DATE	TIME	SERUM SODIUM PER LITER	SERUM CHLORIDE PER LITER	SERUM K PER LITER	HEMOGLOBIN	RED BLOOD CELL VOLUME	R. B. C.	UREA	SUGAR	PULSE	BLOOD PRESSURE	WEIGHT	DISTILLED H_2O INTAKE	URINE VOLUME	URINE SODIUM	URINE CHLORIDE	FLUID RETAINED	REMARKS
A. Adrenalectomized dog—recovery on salt																		
	hours	*m.-eq.*	*m.-eq.*	*m.-eq.*	*grams per cent*	*per cent*	*mil.*	*mgm. per cent*	*mgm. per cent*	*min.*	*mm. Hg*	*kgm.*	*cc.*	*cc.*	*m.-eq.*	*m.-eq.*	*cc.*	
11/25/36	9:00 a.m.	143.7	110.8	6.13	10.5	42.8	5.80	31.7	81.5	88	110	10.6						Normal
11/25/36	12:50 a.m.	125.5	100.0		10.5	37.4	5.76						1100	178			922	Depressed
11/25/36	7:00 p.m.	118.7	89.2	5.81	12.0	46.6	6.0	25.6	91.5	100	104		1690	358	19.1	18.4	1232	Convulsions saline intrav.*
11/26/36	9:40 a.m.	141.8	116.8	6.91	10.5	35.5	5.32	27.7	87	96	98	10.5		685	26.4	20.9		Normal
B. Adrenalectomized dog—primed with extract																		
12/28/36	9:30 a.m.	138.1	111.8	5.60	13.0	41.7	6.80	36.3	82.0	68	106	10.4						Normal extract†
12/28/36	2:20 p.m.	125.3	97.8	4.35	11.9	41.8	6.48						1040	373			668	No symptoms
12/28/36	8:45 p.m.	124.8	94.8	4.02	10.8	35.3	5.94	21.7	82.0	72	102		2600	1773	6.62	18.2	827	No symptoms
12/29/36	9:30 a.m.	136.4	107.2	5.25	11.5	34.5	6.00	24.3	82.0	62	100	10.3		640	0.54	3.28		No symptoms
C. Adrenalectomized dog.—Primed with extract.—Low serum Na and Cl																		
10/27/36	9:30 a.m.	133.0	100.8	5.6	10.3	34.7	4.80	42.5	98	116	86	7.7						Appears normal‡ extract§
10/27/36	4:30 p.m.	119.5	89.4	7.1	7.8	26.9	4.68	25.0	102.5	104	80		1000	580			420	No symptoms
10/27/36	11:05 p.m.	117.6	87.8	7.3	7.4	24.8	3.88	15.7	93.5	100	84		1600	1095	3.54	17.3	505	No symptoms

* 20 cc. of 20 per cent sodium chloride intravenously.

† 3 cc. per kgm. intravenously at 9:30, 1.5 cc. per kgm. at 2:20, 1.5 cc. per kgm. at 6:00 p.m.

‡ Previously on a subminimum maintenance dosage.

§ 3 cc. per kgm. intravenously at 8:30, 1.5 cc. per kgm. at 12:30, 1.5 cc. per kgm. at 6:00 p.m.

symptoms such as hypersensitiveness to mechanical or auditory stimuli, dilated pupils and slight weakness of the hind legs. They were then injected intravenously with 20 cc. of a 20 per cent salt solution. Marked

evidence of blood dilution appeared. A copious urine flow resulted and the symptoms disappeared. Some hours later, following continued forcing of water, slight symptoms returned and were again abolished by injection of salt. The dogs received large volumes of water but drained most of it off in the urine after the salt injections. The high salt intake previous to water administration apparently did not help materially in warding off symptoms. However, the salt was highly efficacious when given by vein. It has been known for a long time that water intoxication can not be induced in normal animals when isotonic salt solution is used.

The effect of priming the adrenalectomized dog with large doses of cortical hormone previous to forcing fluid. The data obtained from study of a representative case are given in table 2B. This type of experiment proved to be highly significant since it was found that by priming the adrenalectomized dog with large doses of salt-free cortical hormone just previous to forcing fluids, the animal remains free from all symptoms of water intoxication. The total fluid intake per kilogram of body weight was far in excess of that of the non-primed dog at the point of convulsions. The serum sodium and chloride dropped during the period of water administration. The blood showed evidence of dilution as revealed by erythrocyte count, hemoglobin and hematocrit determinations. The primed animal is thus comparable in this respect to the normal dog and the animal injected with hypertonic saline. A constant flow of urine was maintained and dilution of the serum persisted. The kidney drained off the excess body fluids almost as rapidly as they accumulated. No significant changes occurred in the arterial pressure or blood glucose but the blood urea nitrogen fell sharply. Approximately twelve hours after the last dose of water was given the serum sodium had risen to within 2 m.-eq./l of the normal and the serum chloride had returned to within approximately 4 m.-eq./l of normal. These primed dogs remained active and vigorous throughout the experiment. Priming with salt-free cortical hormone was, under the conditions of our experiments, as effective in maintaining the animals free from symptoms of water intoxication as priming with hypertonic saline. The serum sodium and chloride of the primed animals do not decline to as low levels as observed in the non-primed adrenalectomized dog, but correspond to those levels characteristic of the normal intact dogs during the convulsive phase. The return to normal level of these serum electrolytes after discontinuing the water administration and in the absence of any food intake, is likewise characteristic of the intact animal. The primed adrenalectomized dog also behaves more like the intact dog than the non-primed adrenalectomized animal in respect to the quantity and concentration of sodium eliminated in the urine. These dogs excrete large volumes of extremely dilute urine containing practically no sodium. It would appear that the cortical hormone exerts some action on the kidney and holds the

renal excretion of sodium in check (compare table 2B with 1A). These results are comparable to those obtained by Harrop et al. (1933) on adrenalectomized dogs during recovery from adrenal insufficiency on large doses of extract.

The effect of priming the adrenalectomized dog on a sub-minimum maintenance dose of hormone and with low serum sodium and chloride levels with large injections of cortical extract. The animals comprising this set of experiments were given a sub-minimum maintenance dose of cortical extract for a period of six days previous to use. The daily dose was just sufficient to keep them free from obvious symptoms, but insufficient to maintain normal levels of serum sodium and chloride, blood urea nitrogen and arterial pressure (table 2C). Such animals rapidly develop fatal water intoxication on relatively small quantities of water. However, when primed with intravenous doses of cortical hormone, previous to forcing water, the dogs remain free from symptoms and retain normal activity and vigor throughout the experiment, despite extremely low levels of serum sodium and chloride. The blood was markedly dilute at the termination of the experiment. The urine contained considerable quantities of chloride but small amounts of sodium.

Discussion. The writers have presented evidence (Swingle, Pfiffner, Vars and Parkins, 1934; Parkins, Taylor and Swingle, 1935; Swingle, Parkins, Taylor and Hays, 1936) from which it seemed clear that the adrenal cortical hormone is an essential factor in the regulation and control of *internal* distribution of body water. The experiments clearly indicate that the restorative action of cortical hormone upon the adrenalectomized dog prostrate from adrenal insufficiency, from sodium and chloride depletion by intraperitoneal glucose, as well as certain other conditions which originate from upsets in osmotic equilibrium, was effected by a redistribution of fluids from intracellular to extracellular compartments (see also Darrow and Yannet, 1935; Gilman, 1934). With adequate hormone present this fluid shift occurred (serum volume and arterial pressure increase and symptoms disappear) in the absence of external sources of fluids or electrolytes, and also at low serum sodium and chloride levels. However, these electrolytes were repeatedly observed to increase in concentration during the period of greatest expansion of serum and other extracellular fluid volume. Calculations show that to maintain the serum sodium level apparently unchanged, in the presence of such marked increase in volume of the extracellular fluid requires a considerable increase in total sodium or else the concentration would decrease.

The data from each set of experiments on water intoxication reveal that during the recovery interval the serum sodium and chloride rise sharply. In the case of the adrenalectomized dog primed with hormone and the intact animal these electrolytes return to normal serum concentration within

a few hours after discontinuing water administration. This raises the question as to the source of these ions. It is our opinion that the increase of sodium and chloride in the serum and other extracellular fluid is from intracellular sources both in water intoxication and in the experiments referred to above. Such a redistribution of sodium and chloride, and possibly other osmotically active substances (e.g. potassium—Harrop, 1936) offers a plausible explanation for: 1, the shift of intracellular water to restore extracellular fluid volume, blood dilution and arterial pressure in the earlier experiments, and 2, the release of the excess accumulation of intracellular water in the present experiments on water intoxication. The experiments on sodium chloride administration demonstrate that an extracellular increase of these ions is sufficient to bring about the described fluid redistribution.

SUMMARY

Normal dogs spontaneously recover from water intoxication if fluid administration is discontinued at the time of the onset of convulsions. The serum sodium and chloride decline to low levels but return to normal during the recovery interval in the absence of food or salt.

Healthy, vigorous, adrenalectomized dogs subjected to water intoxication do not recover unless injected intravenously with either salt-free cortical hormone or hypertonic saline solution.

Adrenalectomized dogs primed with large doses of cortical hormone do not develop water intoxication but the serum sodium and chloride decline to low levels. Serum potassium changes are slight. Similar animals primed by intravenous injections of hypertonic saline solution do not develop water intoxication.

The return of water intoxicated animals to normal health and vigor is associated with a sharp rise in the level of the extracellular electrolytes. It is suggested that the cortical hormone mobilizes and redistributes the intracellular fluid by shifting small amounts of intracellular sodium and chloride (and probably potassium) to extracellular spaces and blood stream.

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FIBER ACTION POTENTIALS IN THE SPINAL CORD OF THE CAT

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Received for publication January 20, 1937

Although a considerable amount of work has appeared on the electrical properties of peripheral nerve, the fiber tracts of the central nervous system have thus far received relatively little attention. Gasser and his associates Graham and Hughes (2) (5) (6) have followed the fibers of the posterior roots into the spinal cord, but it still remains to apply their methods to axones lying entirely within the central nervous system. The present communication relates to the potentials obtained from the cervical cord of the cat after the application of single break shocks to the ventral surface of the medulla.

Under ether anesthesia the basal portion of the occipital bone was exposed by a ventral approach through the neck. Just between the auditory bullae a trephine hole 5 mm. in diameter was made in the bone. Thus the two pyramidal tracts were exposed as they lay on the ventral surface of the medulla at about the level of the seventh nerve. Stimuli were applied by means of a bipolar electrode in which the two silver contacts were set flush into the end of a bakelite rod. This rod could be screwed tightly into the trephine hole in such a way that the anode rested on the ventral surface of one pyramid, the cathode on the other. The insertion of the stimulating rod could be performed entirely without blood loss. Once in place it greatly retarded leakage of cerebrospinal fluid and maintained a relatively constant contact with minimal disturbance to the temperature or blood supply of the medulla. Leads were obtained by means of coaxial needle electrodes which were inserted into the cord through small drill holes in the ventral surfaces of the bodies of the cervical vertebrae. In each experiment two leads were employed, an upper one (lead 1) 17 to 30 mm. caudal to the point of stimulus, and a lower one (lead 2) 20 to 50 mm. caudal to the first. The potentials obtained were visualized by means of the cathode ray oscillograph.

The stimuli consisted of single break shocks from a Harvard inductorium

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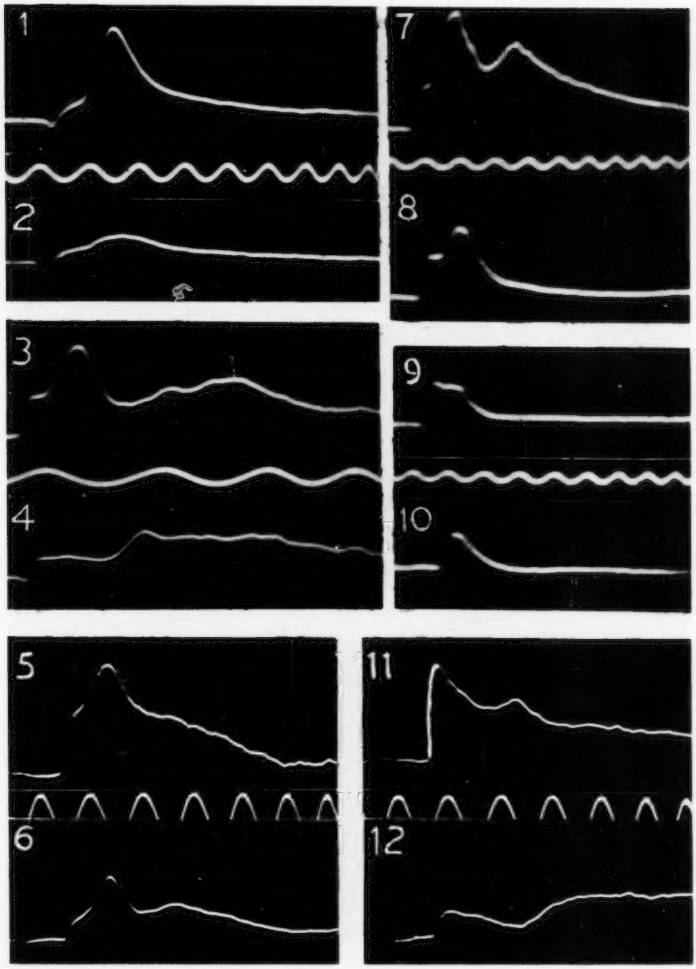
with the secondary in maximum position. Gradations of stimulus strength were obtained by introducing resistance into the primary circuit. After the setting up of each experiment had been completed, the position of the stimulating electrode was adjusted so that a 60 cycle volley produced movement in at least one extremity. The hind legs were found to have a threshold lower than the fore-legs, movement being obtained at about one volt of stimulus strength. Shunting of current by the cerebrospinal fluid was probably very great. Leads were adjusted by pushing them into the cord until maximal potentials were obtained. The needles lay usually at the level of C_2 (lead 1) and C_5 (lead 2). Farther than this from the point of stimulus the potentials were too dispersed for convenient registration. There was no evidence that at a given level, a lead was more effective in one side of the cord than in the other. The shock artifact in some cases was so large that it obscured the start of the resulting potential. However, ten separate determinations were obtained in which accurate measurement was possible.

In all experiments the form of the first negative potential was essentially the same. Figure 1 shows a typical potential which bears a striking resemblance to the classical A spike of peripheral nerve. The rise occupies 0.3 millisecond which is slightly slower than that of the A spike, the latter being circa 0.14 millisecond. The duration of the potential is somewhat longer than that of the A spike, being approximately 0.75 millisecond as opposed to 0.43 millisecond for the latter (3). Notwithstanding these divergences the potential was thought to be in reality comparable to those led from peripheral nerves and the result of the presynaptic activity of fiber pathways within the medulla and spinal cord. This conclusion was based upon its form, already referred to, its resistance to asphyxia, its rate of propagation.

The effect of asphyxia may be seen in the following experiment: Figure 7, registered with a slow oscillograph sweep, shows the first negative potential followed by a second negative complex. Figure 8 depicts the same potential 2 minutes after the animal had been killed by an injection of ether into the heart. It will be noted that the second potential has disappeared while the first is still apparent. In another 2.5 minutes (fig. 9) the first potential is greatly reduced, and 5 minutes following the injection nothing is apparent but the shock artifact (fig. 10). Obviously the first potential is more resistant to asphyxia than the second. Figure 2 is taken from the same experiment as figure 1 and shows the same potential 10 minutes after the exsanguination of the animal through the heart. It is considerably reduced but still clear. Such evidence points to this first complex as a presynaptic fiber potential as opposed to the later possibly postsynaptic potentials which are more susceptible to asphyxia. This is in accord with the observations of Gasser and Graham, who on stimulation



Figs. 1 and 2. Potential obtained from lead 1, before and ten minutes after exsanguination of animal. Time: 2 cycles = 1 millisecond.

Figs. 3 and 4. Potentials from leads 1 and 2, experiment 3. Conduction time between leads 0.51 millisecond, conduction distance 55 mm., conduction rate 108 meters per second. Time: 1 cycle = 1 millisecond.

Figs. 5 and 6. Potentials from lead 1, experiment 2a and b, with stimulus strength of 19 ohms and 69 ohms respectively. Time: 2 cycles = 1 millisecond.

Figs. 7, 8, 9, 10. Effect of ether injection into heart. Potentials before and 2, 4.5, and 5 minutes after injection. Time: 1 cycle = 1 millisecond.

Figs. 11 and 12. Potentials from lead 2, experiment 2a and b, with stimulus strength of 19 ohms and 69 ohms respectively. (Shock artifact retouched in fig. 11.)

of the posterior roots found two potentials exhibiting the same characteristics as those just described. The first they thought to be due to the activity of the incoming fibers in the posterior columns and the second to intrinsic postsynaptic activity of the cord.

The last reason for regarding the first negative complex as a fiber potential is its rapid rate of propagation. Figures 3 and 4 are taken from a single experiment and show typical potentials obtained from leads 1 and 2 respectively. It is readily apparent that the potential from lead 2 which lay 55 mm. downstream from lead 1 comes about 0.5 millisecond later in time. Since at any given level changing the position of the electrodes resulted in no shift of the action potential it was concluded that the lead electrodes were incapable of localizing the responses of different pathways. It seems apparent therefore that the two leads actually recorded the same group of potentials at different spinal cord levels. This conclusion was further supported by the fact that in the majority of cases the conduction velocities were essentially the same calculated from the point of stimulus to either of the leads. Some striking inconsistencies, however, were observed. In experiments 1b, 3, 5a, the conduction velocities computed from the point of stimulus to the first lead were excessively high when compared with the velocities determined from the stimulus to the second lead, or from the first lead to the second. There were two main sources of this obvious error. The first lay in the shortness of the conduction time which in these instances did not allow the first negative potential to become entirely distinct from the shock artifact, and thus made accurate measurement difficult. The second source of error resulted from the fact that it was impossible to determine exactly at what point the stimulus had been effective. This was due to the relatively large surface of the stimulating electrode and to the possibility that the stimulus had jumped. Since the conduction distance from stimulus to lead 1 was usually small, a slight error in measurement resulted in a large discrepancy in calculated conduction velocity.

In the case of lead 2, however, these small errors were less important because of the longer distances involved. For the most reliable determinations it was necessary to utilize the accurately measurable distance between the two leads as the basis for velocity calculations. Hence the conduction rates recorded in the first column of table 1 may be regarded as the most significant. It will be seen that the velocities calculated to lead 2 are in close agreement with these. The first negative potential was thus found to be propagated at rates varying between 59 and 112 meters per second. Such rates fall easily within the range reported for peripheral nerve (1), (3), (4), the maximum velocity of 112 m/sec. being slightly greater than the maximum of 100 m/sec. usually given for peripheral nerve.

Study of the conduction times in table 1 indicates that the potentials in question are the record of impulses which have not traversed a synapse. For, with conduction distances from 17 to 85 mm., the range of conduction times is from 0.15 to 1.0 millisecond which is well below that of 0.5 to 1.36 milliseconds assigned by Lorente de Nó (7) for synaptic delay alone.

It was therefore concluded that these potentials do in reality represent the presynaptic activity of nerve fibers, and in this sense can be considered as true spikes. Care must be exercised, however, in pushing further the analogy with the peripheral nerve. One can see from table 1 that in general, the stronger the stimulus the more rapid the propagation of the resultant potential (the lower the resistance reading the stronger the stimulus). For example, figures 6 and 12 show a potential obtained from

TABLE 1

EXPERIMENT	STIMULUS STRENGTH	LEAD 1-2			STIMULUS—LEAD 1			STIMULUS -LEAD 2		
		C. T.	C. D.	C. R.	C. T.	C. D.	C. R.	C. T.	C. D.	C. R.
	ohms									
1a	19	0.42	47	112	0.18	20	111	0.60	67	110
2a	19	0.41	45.5	111	0.15	17	113	0.56	62.5	111
3	19	0.51	55	108	0.20	30	150	0.71	85	120
4	59	0.31	29	93	0.32	27	85	0.63	56	89
5a	59	0.35	31	89	0.19	24	126	0.54	55	105
5b	179	0.41	31	77	0.31	24	76	0.72	55	77
2b	69	0.60	45.5	76	0.20	17	85	0.80	62.5	78
6	77	0.40	30	75	0.25	18	72	0.65	48	74
7	99	0.43	32	74	0.27	18	67	0.70	50	71
1b	79	0.80	47	59	0.20	20	100	1.00	67	67

C. T., conduction time in milliseconds; C. D., conduction distance in millimeters; C. R., conduction rate in meters/second.

leads 1 and 2 respectively, with an arbitrary stimulating strength of 69 ohms (expt. 2b). The velocity of the most rapidly conducting fibers, calculated between the two leads, is 76 meters per second. When the stimulus strength is increased to 19 ohms (figs. 5 and 11, expt. 2a), the velocity rises to 111 meters per second. This direct correspondence between increasing stimulus strength and conduction velocity appears superficially to be quite the reverse of peripheral nerve where the more rapid fibers have the lower threshold. However, when the potential from lead 1 obtained with the weaker stimulus (fig. 6) is compared with that resulting from the stronger stimulus (fig. 5) a new fact comes to light. Not only does the potential develop more rapidly after the stronger shock, but it also increases in height and duration. This finding indicates that fibers representing many different conduction velocities have been ac-

tivated by the stronger stimulus. In contrast to peripheral nerve it was not possible in these experiments to bring in any individual velocity group alone.

The reason for this is not far to seek. With its small size the peripheral nerve is in effect equally and completely stimulable from any point on its circumference. This is not true of the larger medulla and spinal cord where, in addition to the factor of unequal accessibility to the stimulus, the cerebrospinal fluid introduces more complicated shunting effects. At the outset it was thought that since the stimulus was applied directly to the pyramids with resulting muscular contraction, the potential obtained represented that of the cortico-spinal tract. There was, however, no visible modification of the complex in an animal whose cortico-spinal tracts had been brought to complete degeneration by removal of the electrically excitable cortex. It is therefore impossible to speak of this rapidly propagated potential as the response of any given anatomical pathway. In spite of its spike-like form it appears rather to be a very complex phenomenon representing the presynaptic activity of whatever fibers happen to be accessible to the stimulus under the conditions of a given experiment. Thus when the strength of the stimulus is increased, many new fibers of various types are brought into range, and the area of the already multifarious potential is increased in all directions. The correlation between conduction velocity and "threshold" observed by electrophysiologists in peripheral nerve is thus not demonstrable in the spinal cord with the technique employed.

The authors wish to express their gratitude to Dr. Herbert S. Gasser for his interest in the work and his generosity in extending to them the facilities of his laboratory.

SUMMARY

1. Single break shocks from an induction coil were applied directly to the ventral surface of the cat's medulla and the resulting potentials were led from the cervical cord.

2. Because of their form, resistance to asphyxia, and rapidity of conduction (59–112 m/sec.) these potentials are thought to represent presynpatic fiber activity.

3. Although the potentials described are considered in a sense analogous to the spikes of peripheral nerve, the physical properties of the cord prevented the establishment of velocity groups according to threshold. Consequently the responses obtained from the cord were very complex and doubtless represented the activity of many fiber pathways.

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HEME CONTAINING FRACTIONS OF BLOOD AS RELATED TO THE AUGMENTATION OF PITUITARY GONADOTROPIC EXTRACTS[1]

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Received for publication January 20, 1937

Extracts of various substances such as urine and blood serum have been shown by Evans, Simpson and Austin (1), Cole and Hart (2), Saunders and Cole (3), Freed (4) and Hellbaum (5) to increase the action of anterior pituitary gonadotropic preparations. Maxwell (6) has reported that zinc sulphate, and Fevold, Hisaw and Greep (7) that zinc and copper salts, yeast extract and yeast ash produce a similar effect. Kraatz (8) has shown that leucocytes, and Casida (9) that the formed elements of blood augment gonadotropic extracts and that the ash from the formed elements does not augment.

The fact that copper and zinc salts (6), (7) cause augmentive action while the ash (inorganic) from the formed elements of blood (9) does not augment made it of interest to determine what substance or substances contained in the formed elements of blood is responsible for the augmentation of pituitary gonadotropic extracts. Therefore a study was made of the augmentive action of cow blood and of erythrocytes, hemoglobin, globin, porphyrin, heme and hemin.

EXPERIMENTAL METHODS. The gonad-stimulating extracts used in the experiments to be reported were of two types, a 33 per cent acetone soluble fraction of a pyridine extract of whole desiccated sheep pituitary gland made by the method reported by Fevold, Hisaw, Hellbaum and Hertz (10) and a water soluble fraction prepared by extracting whole desiccated sheep gland with water at pH 6.5 to 7.0.

The hemoglobin was prepared from fresh citrated cow blood by the method of Johnson and Bradley (11) which involved the treatment of washed erythrocytes with freshly prepared aluminum hydroxide and toluene. Heme and globin were prepared from the hemoglobin (11) by treating it with 0.1 N HCl in the cold and the addition of this mixture to cold acid acetone which dissolved the heme and precipitated the globin. Since it seemed probable that this method of preparation might not yield

[1] This work was supported in part by a grant from the Wisconsin Alumni Research Foundation.

pure heme, crystalline hemin hydrochloride was prepared according to the method used by Drabkin and Austin (12) and washed three times with water and twice with 65 per cent alcohol. The blood porphyrin was made from washed erythrocytes by the method of Papendieck (13). By the use of the above methods the blood substances already mentioned were separated so that their ability to augment the gonadotropic extracts could be tested.[2]

These determinations were made by mixing in vitro a given quantity of each substance with a definite amount of the gonadotropic extract and by injecting the mixture subcutaneously into immature female rats 21 days old. The rats were injected with 0.5 cc. during the afternoon of the first day and twice daily with this amount on each of the four succeeding days. The animals were killed during the morning of the fifth day and the ovaries removed, examined before a strong light for the presence of relative numbers of follicles and corpora lutea, and then weighed.

Two cubic centimeters of whole blood, or its equivalent of erythrocytes, hemoglobin, globin, heme, hemin and porphyrin were used consistently in testing for augmentive action. The iron salts were given in quantities (5 mgm.) containing about twice the amount of iron found in the hemin from 2 cc. of cow blood. As hemin is not soluble in water it was necessary to add 0.5 per cent sodium carbonate to insure its solution. To this solution was added the gonadotropic extract and enough water so the total volume for injection was 4.5 cc. per rat which made the concentration of sodium carbonate 0.1 per cent. The concentration of the sodium carbonate and the volume of the control solution (gonadotropic extract without augmenter) was made to equal that of the mixture of augmenter and extract. In certain of the experiments the augmenter (hemin) and the gonad-stimulating extract were not mixed but each was injected separately and subcutaneously into a different part of the body of the rat. In these cases the solutions for injection were prepared as already described and 0.25 cc. of each was injected twice daily for 4.5 days.

Results and discussion. The results obtained by the use of the procedures described are summarized in table 1. The difference between the mean ovarian weight obtained with the injection of the pituitary extract alone and the mean weight of the ovaries from the rats which received the same quantity of extract plus the augmenter is considered as the amount of augmentation produced. The results demonstrate that whole blood, hemolyzed erythrocytes, hemoglobin, heme and hemin augment the activity of gonadotropic extracts prepared from the anterior pituitary glands of sheep. The augmentation produced by whole blood, erythrocytes, and hemoglobin

[2] The term "augmenter" as used in this report designates the substance that was mixed and injected with the gonadotropic extract regardless of whether or not the substance enhanced the effect of the preparation.

TABLE 1

Comparison of the ovarian weights of immature rats receiving gonadotropic extracts of the anterior pituitary gland with those receiving the extracts plus augmenters

GONADOTROPIC EXTRACT		AUGMENTER		NUMBER OF RATS	M. ± S.E.*	C. V.*	M. DIFF. ± S.E.*	P*
Kind	Amount	Kind	Amount					
					mgm.		*mgm.*	
				23	30± 4.0	63.4		
		Whole blood	2 cc.	6	141±14.3	24.8	111±10.5	<.01
		Blood cells	2 cc. equiv.	9	93±12.7	41.0	63±10.1	<.01
		Hemoglobin	2 cc. equiv.	18	70± 8.4	51.0	40± 8.6	<.01
		Globin	2 cc. equiv.	21	58± 8.8	72.0	16± 9.3	>.05
33 per cent acetone soluble	100 mgm. equiv. dry tissue	Porphyrin	2 cc. equiv.	6	21± 3.1	36.8	7± 8.0	>.05
		Ferrous chloride	5 mgm.	5	17± 1.6	21.8	13± 8.7	>.05
		Ferric chloride	5 mgm.	3	25± 5.3	36.8	5±11.4	>.05
		Heme	2 cc. equiv.	13	175±16.2	33.5	145±13.2	<.01
		Hemin	8 mgm.	6	223±26.4	29.0	193±15.0	<.01
				10	70±10.4	47.1		
Water soluble	100 mgm. equiv. dry tissue	Ferric chloride	5 mgm.	6	62± 4.6	18.0	8±14.0	>.05
		Hemin	8 mgm.	7	213±22.7	28.2	143±22.4	<.01
				12	40± 6.2	51.2		
Water soluble	50 mgm. equiv. dry tissue	Hemin	4 mgm.	4	213±49.2	46.2	173±28.2	<.01
		Hemin†	4 mgm.	9	25± 4.0	47.6	15± 7.6	>.05

* M ± S.E. indicates the mean ovarian weight and its standard error; C.V. the coefficient of variation; M. diff. ± S.E., the difference and its standard error between the mean ovarian weight obtained with the gonadotropic extract alone and that with the extract plus the augmenter. P indicates per cent of cases in which the difference occurred from sampling and when it is less than 0.01 it is considered highly significant, when greater than 0.05, not significant.

† The hemin and gonadotropic extracts were not mixed but each was injected separately.

is probably a result of the breakdown of these substances into globin and heme after which the heme combines with the pituitary substance to cause the augmentation. The ovarian weights obtained with whole blood plus the extract (141±14.3 mgm.) are significantly greater than were obtained with the same equivalent of hemolyzed erythrocytes or hemoglobin plus the pituitary extract (93±12.7 mgm. and 70±8.4 mgm. respectively). When this extract was given in the same quantity without the augmenter, it gave an average ovarian weight of 30±4.0 mgm. This difference in the ovarian weights obtained with whole blood, erythrocytes and hemoglobin is in part, no doubt, a result of the inability to obtain a one hundred per cent recovery of all the hemoglobin and erythrocytes from a given sample of blood. In addition there is some evidence (Casida, 9; Cole and Hart, 2; Saunders and Cole, 3) that serum has an augmentive action which probably accounts for a part of the above differences.

The heme prepared from hemoglobin and injected with the gonadotropic extract in an amount equivalent to 2 cc. of blood gave a greater increase in ovarian weight (175±16.2 mgm.) than an equivalent amount of hemoglobin (70±8.4 mgm.). The greatest increase in the mean ovarian weight (223±26.4 mgm.) was obtained when 8 mgm. of hemin were given with the extract.[3] This represents the amount of hemin contained in 1.5 to 2 cc. of cow blood. The globin prepared from hemoglobin did not give significant augmentation when injected with the extract in quantities equivalent to 2 cc. of blood (58±8.8). This indicates that the augmentation produced by the hemoglobin depends upon the heme part of the pigment. It was demonstrated that when the globin was added to the gonadotropic extract precipitation occurred, which was probably the result of the combination of the globin with protein present in the extract. Bischoff (14) has also shown that thymus histone precipitates insulin, and certain substances associated with prolan and pituitary gonadotropic extracts.

Since heme contains iron in organic combination, iron in the inorganic form was tested for its augmentive action. The average weight of the ovaries (17±1.6 mgm. and 25±5.3 mgm.) obtained from rats injected with the gonadotropic extract plus the iron was not as large as the average weight (30±4.0 mgm.) of those from rats which received the extract alone, which demonstrates that inorganic iron does not augment. These results confirm those reported by Fevold, Hisaw and Greep (7). Another series of rats was treated with the extract plus the porphyrin prepared from cow blood. Again there was no significant difference between the weight of the ovaries from the control rats on the extract alone (30±4.0 mgm.) and the weight of the ovaries from the rats treated with the por-

[3] In a quantitative study of the augmentive action of heme using different amounts of the augmenter and pituitary extract it was found that 4 mgm. of hemin were approximately as effective in producing augmentation as 8 mgm. (unpublished data).

phyrin plus the extract (21±3.1 mgm.). When heme was injected alone there was no increase in the weight of the ovaries of immature female rats. These results indicate that this augmentation depends upon the organic combination of iron with porphyrin as represented in the heme.

When the augmenter (hemin) and the gonadotropic extract were injected separately the mean ovarian weight was 25±4.0 mgm., that for rats injected with gonadotropic extract only, 40±6.2 mgm. Whereas, when the hemin was mixed in vitro with the gonadotropic extract the mean ovarian weight was 213±49.2 mgm. Since the separate injection of hemin and gonadotropic extract did not result in augmentation and the in vitro mixture did, it is suggested that there may be a combination of the heme with the gonadotropic complex in vitro which is not formed in the body of the rat when they are injected separately. Perhaps there is formed in the in vitro mixture a "hemochromogen-like" compound which is considerably more active than the gonadotropic complex. Krebs (15) has shown that heme is a very poor catalyst but when combined with certain nitrogenous groups it forms hemochromogens, some of which are very effective catalysts, the increased catalytic power of the heme depending upon the substance with which it is combined.

The explanation of the augmentive action of heme when mixed in vitro with pituitary extracts is not known. However, it does not seem that the delay of absorption as has been suggested for zinc salts will account for the augmentive action of heme as there is no evidence of the formation of an insoluble gonadotropic complex when the heme is mixed with the extract. The addition of globin to gonadotropic extracts produces a precipitate but there is no augmentation when the mixture is injected. Therefore the formation of insoluble precipitates and augmentation are not necessarily associated.

CONCLUSIONS

Whole blood, hemolyzed erythrocytes, hemoglobin and heme greatly augment the effect of anterior pituitary gonadotropic extracts on the ovaries of immature rats when mixed before injection.

Porphyrin and globin prepared from hemoglobin and ferrous and ferric chloride do not have any augmentive action.

Augmentation is not obtained when the heme or whole blood is injected separately and in such a way that there is not mixing before absorption.

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PHASIC BLOOD FLOW AND ITS DETERMINANTS IN THE RIGHT CORONARY ARTERY

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Received for publication January 22, 1937

Although phasic blood flow and its determinants has been extensively studied in branches of the left coronary artery (1), the flow through the right coronary has not received the attention that it deserves The important observations, that in the heart-lung preparation approximately 40 per cent of right coronary inflow drains into the coronary sinus (2), and that in the fibrillating heart the right coronary empties almost entirely through the Thebesian vessels of the right ventricle (3), offer but meager insight into the ultimate mechanisms concerned with right coronary blood flow.

It would be expected that the aortic or central coronary pressure would play the dominant rôle in influencing right coronary inflow. This is so not only because it constitutes the driving pressure tending to force blood into the coronary vascular bed but also because its ordinate value is far in excess of right ventricular pressure (and hence probably of myocardial resistance to flow in the right heart).

Counteracting or aiding such a driving pressure are a number of peripheral factors which may affect blood flow through the right coronary such as *a*, the phasic resistance to flow in the right ventricle; *b*, the anatomical connections between the right and left coronaries; *c*, distribution of the right coronary to the auricles and to portions of the left ventricle, and *d*, the fact that the right coronary has its venous outlet in both the right ventricular chamber and the coronary sinus.

Since these possibilities have not heretofore been experimentally evaluated, a study of right coronary inflow was therefore undertaken.

PROCEDURE. The method and apparatus employed were quite similar to those previously used in measuring the volume and velocity of blood flow into the left coronary artery (4, 5, 6). In brief this method depends on determining the pressure difference between the aorta and the peripheral end of a coronary artery at various stages of a cardiac cycle, when flow in the coronary vessel is prevented by temporary and permanent clamping of the vessel central to the point of measurement. The height of such a

differential pressure curve at any point gives velocity of flow and the area under any portion gives volume flow.

All experiments were performed on anesthetized dogs with chest open. The right coronary artery, near its origin, and either its terminus or a side branch were isolated. The aortic, right ventricular and central and peripheral coronary pressure curves, together with the maximum right coronary systolic resistance were then recorded utilizing optical manometers (Wiggers type) and clamping devices already described (5). In addition, in some of the experiments the pressure variations were taken by a modified form of the Hamilton manometer (8). To study the relationship between right ventricular pressure and right coronary inflow, the right ventricular pressure was raised by a special clamp on the pulmonary artery (9) while the aortic pressure was kept reasonably constant through compression of the aorta just above the diaphragm. In all experiments great care was taken to support the heart so that any possible artefacts in the right coronary records might be reduced to a minimum. Despite this it was found exceedingly difficult to obtain records entirely free from error.

Results. Typical records of aortic and right ventricular together with right peripheral coronary pressures taken after centrally clamping the right coronary artery are reproduced in figure 1A, B. In A the aortic pressure is 95/62 mm. Hg and the right ventricular pressure is 35/2 mm. Hg. In the fourth beat after clamping the artery at *X*, the P.C.P. (peripheral coronary pressure) curve is reasonably stabilized. The curve starts to rise at *a* during the right ventricular isometric contraction period and continues upward rather rapidly at first and then more slowly reaching its highest point at *d* late in systole. Most of the rise of the curve occurs after the isometric contraction period, i.e., *b-d*. The curve then declines and attains its diastolic level, *e*, during the time of right ventricular inflow. The pressure is 23 mm. Hg in diastole and rises during systole to 32 mm. Hg. By intermittent systolic clamping of the artery (later in the same record but not shown here), the maximum systolic resistance is found to be 47 mm. Hg. Figure 1B is characterized by a large central pulse pressure, 103/54 mm. Hg induced by saline infusion. The systolic holding pressure obtained in the first beat after clamping the artery at *X*, is slightly less than 42 mm. Hg, while the diastolic resistance in the fourth beat is 22 mm. Hg. These values and the contour of the peripheral pressure curve are similar to those in A.

Some of our records display somewhat different ordinate values and contours but from an examination of a great many curves we feel that the curves in A, B, give a reasonably exact picture of the right peripheral coronary pulse. It is of interest to compare such curves and figures with those previously obtained from branches of the left coronary artery (4, 5, 6).

With minor deviations, the P.C.P. curves from both ventricles have the same general contour, rise and fall at the same time with respect to their ventricular pressures, and agree as to diastolic value. The systolic resistances are, however, markedly different. In branches of the left coro-

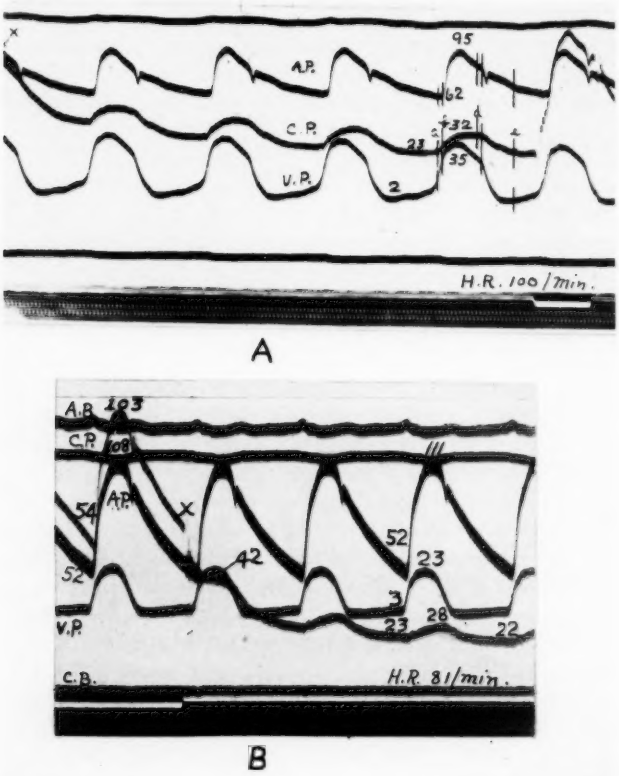

Fig. 1. A, B, records used for determining form of peripheral coronary pressure, the diastolic resistance, and maximum systolic resistance in the right coronary artery. *A.P.*, aortic pressure; *A.B.*, aortic base; *C.P.*, coronary pressure; *C.B.*, coronary base; *V.P.*, right ventricular pressure; *X*, time of clamping coronary artery. Time 0.02 second.

nary, the systolic pressure, approximately 40–50 mm. Hg, obtained by permanently clamping the coronary artery central to the recording manometer and the maximum systolic resistance, approximately 75 mm. Hg, obtained by intermittent systolic clamping of the central portion of the coronary artery, are both much greater in absolute value than in the right

coronary ramus. However, when such pressures are compared with the systolic intraventricular pressures in the respective ventricles, they are relatively far greater in the right coronary. In fact the systolic resistance to flow as measured in the right coronary exceeds by varying amounts the right intraventricular pressure. In figure 1A, B the systolic holding pressures are 10 and 19 mm. Hg greater than the corresponding ventricular pressures.

From the curves and data of figure 1A, B, differential flow curves for the right coronary artery have been reconstructed in figure 2A, B (for

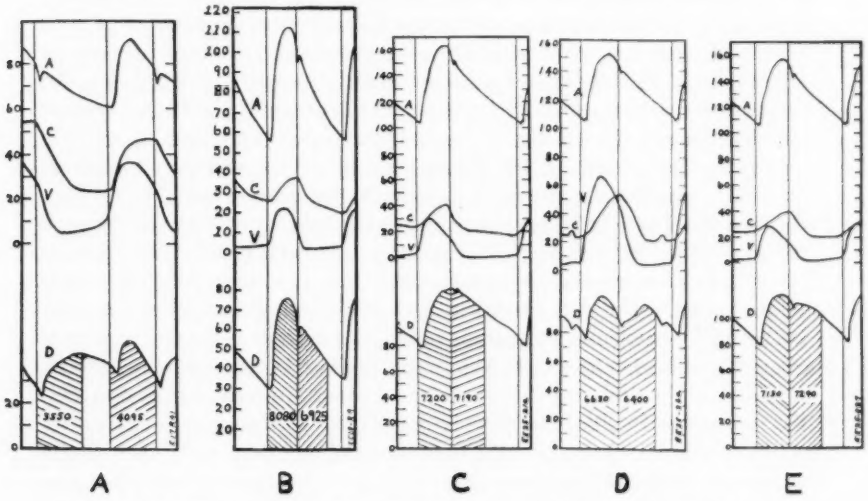

Fig. 2. Charts reconstructed from original records depicting phasic distribution of blood flow in the right coronary artery during single heart cycles. A, B, reconstruction of flow curves from figure 1, A, B. C, D, E, reconstructions showing effect of high right ventricular pressure. *A*, aortic pressure; *C*, peripheral right coronary pressure raised to its true ordinate value; *V*, right ventricular pressure; *D*, velocity curve. Shaded areas, volume flow expressed as square millimeters of area. Ordinates, millimeter Hg.

method see 5). In figure 2A, the velocity curve, D, is very similar to that previously described (5, 6) for the left coronary artery except that here the systolic velocity exceeds the diastolic velocity. In figure 2B the velocity curve is a fairly good reproduction of the aortic pressure curve. In A and B the systolic volume flows are 4095 and 8080 sq. mm. as compared with 3550 and 6925 sq. mm. during an equivalent time interval of diastole. Construction of a considerable number of right coronary flow curves from original records taken under good circulatory conditions indicates that it is rare for the diastolic velocity to exceed the systolic and that

the systolic volume flow ranges from 80 to 120 per cent of the diastolic for an equivalent time period.[1] Such a relation between systolic and diastolic blood flow contrasts with that obtainable in the left coronary in which the systolic flow is rarely more than 75 per cent of the diastolic flow.

We were next interested in observing the effect of augmentation of right ventricular pressure upon the phasic and total blood flow per cycle.

The right ventricular pressure was raised by graded compression of the pulmonary artery just beyond the pulmonary conus, while the right ventricular, aortic and central and peripheral right coronary pressures together with the maximum systolic resistance were being recorded. Such curves and data have been used to establish the flow curves of figure 2C, D, E. In figure 2C with a heart rate of 65 per minute and an aortic pressure of 162/105 mm. Hg and with a right ventricular pressure of 29/2 mm. Hg, the peripheral coronary diastolic resistance is 18 mm. Hg and the systolic holding pressure 42 mm. Hg. The systolic volume flow (7200 sq. mm.) is essentially the same as that (7190 sq. mm.) during an equivalent diastolic interval. In chart D, the right ventricular pressure is raised to 66 mm. Hg, the heart rate is 78 per minute, and the aortic pressure has dropped slightly to 155/105 mm. Hg. The result is that both systolic and diastolic blood flows decrease slightly to essentially the same figure, 6630 and 6400 sq. mm. area. The systolic flow reduction is largely due to the fact that as the right ventricular pressure is raised, the systolic resistance increases from 42 mm. Hg in chart C, to 53 mm. Hg in chart D. A second contributing factor is the drop in aortic or central coronary pressure to 155 mm. Hg. The reduction in diastolic flow is caused by the slightly lower aortic pressure at the incisura and the fact that the coronary resistance at the time of the incisura is higher. The diastolic coronary resistance remains unchanged at 20 mm. Hg.

In chart E, the pulmonary clamp has been released and the right ventricular pressure falls to 27 mm. Hg with a heart rate of 77 per minute and blood pressure of 157/105 mm. Hg. Coincident with the drop in ventricular pressure, the systolic and diastolic blood flows return to 7130 and 7290 sq. mm. area which is approximately the level in chart A. This is due to the fact that with the decrease in right ventricular pressure the systolic resistance decreases to 41 mm. Hg while the diastolic remains at 20 mm. Hg.

Such results which have been obtained many times in favorable preparations indicate that upon elevation of the right ventricular pressure there is

[1] A strict interpretation of the above would lead to the idea that the left coronary artery in the dog carries less blood than the right, since the diastolic resistance is approximately the same while the systolic resistance in the left ventricle is 30 to 40 mm. Hg greater than in the right. It should be obvious that the curves in figure 2 merely give *relative velocities and volume flows* during the heart cycle.

a mild reduction in systolic and diastolic flow. A cursory examination of the above curves indicates that this reduction in flow will invariably be small. Since the time relations of the P.C.P. curve to the central coronary pressure curve are not observed to change while the diastolic resistance is the same, the reduction in flow is due to elevation of the systolic resistance; but since the rise in systolic resistance is generally only a few millimeters Hg (11 in fig. 2D), it can have but little effect on the large effective pressure (113 mm. Hg in fig. 2D) at the height of systole. However the aortic pressures in figure 2 are fairly high and it is obvious that at lower levels of aortic pressure the percentage reduction in systolic flow would be considerably augmented. This state of affairs contrasts with that in the left coronary upon elevation of the aortic pressure (6). In the latter both systolic and diastolic flows are augmented markedly because the systolic and diastolic resistances, although elevated, are increased much less than the aortic or central coronary pressure.

Discussion. From the foregoing, the systolic and diastolic resistances in the right coronary artery, with normal pressures prevailing, exceed the systolic and diastolic right intraventricular pressures by 10–20 mm. Hg. With such levels of systolic and diastolic resistance (approximately 40 and 20 mm. Hg), it is not surprising that the systolic flow in the right coronary generally exceeds the diastolic and that the flow curve also tends to follow the contour of the central coronary or aortic pressure curve, especially when the pulse pressure is large (fig. 2B) or the blood pressure is fairly high (fig. 2C, E).

From the flow curves of figure 2C, D, E it appears that the right coronary inflow is not heavily dependent on the pressure existing in the right ventricle for when the right ventricular pressure was raised to 66 mm. Hg in D, the diastolic resistance remained unchanged and the systolic rose only moderately. Hence the arterial inflow was but little decreased. Normally the venous outflow of the right coronary is fractionated between the channels draining into the right ventricle and the coronary sinus. Apparently with high right ventricular pressures prevailing, the venous blood is shunted, at least partially, from the right ventricular cavity to the coronary sinus, a region of lower resistance (7). Hence under this dynamic state of the right heart, the decrease in coronary inflow which might possibly have been occasioned by the augmented right ventricular pressure is almost entirely compensated for by the increased drainage through the coronary sinus.

Although we believe that such curves give a reasonably accurate picture of arterial inflow into the right coronary, it remains to be determined whether they also represent solely blood flow through the vascular bed of the right ventricle. The right coronary artery not only feeds the right ventricle but also sends branches to auricular muscle and to the left ven-

tricle, regions of lower and higher pressures respectively. In other words, anastomotic connections with other coronary arteries or the possibility of arterial runoff into regions of lower resistance must be definitely ruled out before it can be stated that the velocity and volume curves obtained from the right coronary can also be applied to the right ventricle.

It is common knowledge that in dead hearts anatomical communications between right and left coronary arteries can be demonstrated by injecting under pressure india ink or some similar substance into either artery and observing its subsequent appearance in the other artery. We do not

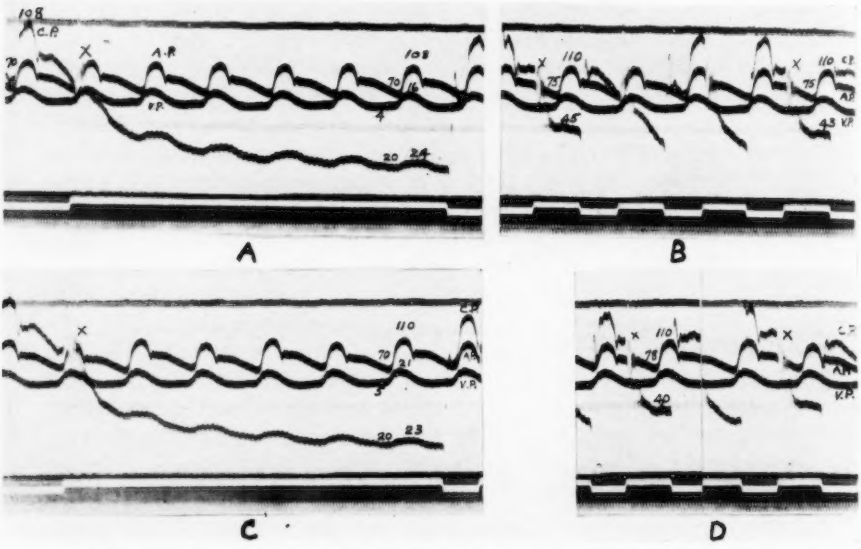

Fig. 3. Records illustrating lack of effect of clamping ramus descendens anterior on peripheral coronary resistance in right coronary. A, control for C; B, control for D. Letters on curves, same as in figure 1.

consider it at all likely that these anatomical connections are communicating ones, for in previous studies on blood flow in the ramus descendens anterior, while either the left circumflex or right coronary or both were clamped, there was no demonstrable change in peripheral coronary resistance (4). However this remote possibility was tested experimentally by recording the peripheral coronary resistance during clampings of short duration of the ramus descendens anterior and/or the left circumflex coronary artery. In some of these preparations gross inspection of the heart disclosed what appeared to be superficial communications between branches of the right coronary and ramus descendens anterior and the existence of

such anatomical connections could be confirmed at the end of the experiment by injection. In figure 3A, B, C, D is shown the lack of effect of clamping the ramus descendens anterior on systolic and diastolic resistances in the right coronary artery. B and A show control values for the systolic holding pressure of 43 mm. Hg and for diastolic resistance of 20 mm. Hg. The left coronary artery was then clamped central to the recording manometer and after approximately three minutes records C, D were taken from the right coronary. The values for peripheral resistances of 20 and 40 mm. Hg are essentially the same as for the controls in A, B.

The same negative results have also been obtained during clamping of the left circumflex either separately or together with the anterior descendens.

In like manner the reverse possibility that the coronary resistance as measured in the right coronary might have been lowered by arterial runoff into right auricular muscle has been investigated. Tying of all visible auricular branches in three experiments did not alter the resistance in the vascular bed.

On the basis of such experiments we feel that the impedance to systolic and diastolic flow in the right coronary comes largely from the right ventricle itself.

A further point of interest is the fact that during systole of the right ventricle, the resistance to blood flow exceeds the existing right ventricular pressure. The only exception to this rule thus far discovered is with quite high right ventricular pressures under which condition the right ventricular pressure generally exceeds the peripheral systolic coronary resistance. The explanation for this apparent paradox may possibly be that the resistance during diastole is a considerable portion of the peak resistance reached during systole, or in other words, systole does not contribute all the resistance observable during this phase of the cardiac cycle. An example will make this clear. In figure 2A the diastolic resistance is 23 mm. Hg as compared with a diastolic level of 2 mm. Hg in the right ventricle. During systole the right intraventricular pressure is raised to 35 mm. Hg whereas the right coronary systolic pressure is raised to 47 mm. Hg or a systolic addition of only 24 mm. Hg as compared with 33 mm. Hg in the right ventricle. At high levels of right ventricular pressure, the maximum systolic resistance will tend to equal or be less than the ventricular pressure since the systolic resistance to flow increases less rapidly than the right ventricular pressure.

SUMMARY

1. Using optical manometers of adequate frequency, phasic blood flow and its determinants in the right coronary artery of the dog have been studied by the method of differential pressure curves.

2. It is found that the P.C.P. curve has a contour similar to that previously described for the left coronary artery. The diastolic ordinate value (15–25 mm. Hg) is also similar but the systolic value (25–30 mm. Hg) is generally lower than the left. The maximum systolic resistance to flow (35–45 mm. Hg) in the right ventricle exceeds the right intraventricular pressure by varying amounts.

3. Apparently most of the intramural resistance to flow resides in the right ventricle for the acute removal of various anatomical connections with the auricles, left ventricle, and other coronary branches does not significantly alter the P.C.P. curve whereas elevation of the right intraventricular pressure increases moderately the systolic holding pressure.

4. With such low levels of peripheral coronary resistance existing, the contour of the velocity curve tends to follow that of the central coronary or aortic pulse while the velocity of blood flow during systole exceeds that during diastole.

5. Based upon equivalent time intervals of systole and diastole, the volume flow during systole generally equals or exceeds that during diastole. If right intraventricular pressure is gradually increased, both systolic and diastolic flow are reduced, the cause of both flow reductions being the elevation of systolic coronary resistance.

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THE METABOLISM OF ADRENALECTOMIZED RATS

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Received for publication January 23, 1937

The influence of extirpation of the adrenal glands upon metabolism has been studied by a number of investigators. The literature up to 1936 has been surveyed by Grollman (1). No satisfactory experiments were found in the literature using rats as experimental animals for total metabolism determinations after adrenalectomy. In the course of the investigations on the metabolism of various carbohydrate substances in the white rat several hundred determinations have been made on this animal (2, 3, 4, 5). The method under the conditions used, presents an excellent opportunity for studying slight changes in metabolism. It was thought, therefore, that a large number of experiments under carefully controlled conditions, treated statistically would give the exact value of the metabolism of the rat after adrenalectomy.

EXPERIMENTAL. With certain minor modifications, Haldane's open circuit apparatus was employed for the determination of CO_2 production, O_2 consumption and R.Q. The details of the method have been described previously (2). Male animals were allowed access to their regular diet (a powder containing whole wheat, casein, klim, NaCl, 1 per cent, $CaCO_3$, 1.5 per cent, and butter fat) at all times. The adrenalectomies were performed by the method described by Grollman (1). Daily records of body weight, food consumption, rectal body temperature and respiratory metabolism at 28° to 30°C. were kept on all animals for 10 days before adrenalectomy and continued after adrenalectomy until death. Control experiments with normal animals on a restricted food intake comparable to adrenalectomized animals were also carried out. Metabolism experiments on adrenalectomized animals were made without a preliminary fasting period. In a preliminary series 9, 48-hour fasting oxygen consumption values were determined and compared with 6 non-fasting values on the same animals. The average value for oxygen consumption per 100 grams of animal after fasting was 0.205 gram per hour as compared with 0.206 gram per hour of non-fasting animals. Further evidence that there is practically no change

[1] Emerson Fellow in Pharmacology.

in O_2 consumption on a restricted diet is shown in 2 experiments carried out on normal animals where the body weight dropped an average of 54

TABLE 1

Condensed table of results

Mean values

RAT NUMBER	NORMAL NON-FASTING ANIMALS				ADRENALECTOMIZED NON-FASTING ANIMALS					
	Body temperature	O_2 consumed, mgm. per 100 grams rat per hour	R.Q.	Calories per sq. m. per 24 hours	Body temperature	O_2 consumed, mgm. per 100 grams rat per hour	R.Q.	Calories per sq. m. per 24 hours	Per cent caloric decrease before death	Surv val period after adrenalectomy
	°F.				°F.					*days*
		227*				244*				
1		199	0.929	1,079	98.2	176	0.889	1,017	−32	33
		182				127				
		210				258				
2		187	1.018	1,038	99.4	209	0.777	1,137	−3	9
		172				174				
		223				197				
3	98.0	209	1.000	1,137	99.6	178	0.772	988	−25	12
		198				154				
		201				183				
4	98.4	176	0.870	1,033	97.5	153	0.903	935	−39	11
		156				109				
		195				156				
5	99.6	159	0.929	986	98.0	137	0.750	800	−28	13
		134				120				
		216				193				
6	99.1	185	0.930	1,100	98.0	164	0.866	974	−23	8
		159				142				
		224				186				
7	98.7	192	0.869	1,085	98.3	179	0.757	1,014	−17	9
		171				165				
		211				174				
8	99.1	173		1,084	98.3	155	0.771	924	−26	8
		150				138				
		214				177				
9	98.9	185		1,062	98.3	158	0.915	928	−30	17
		167				134				
		217				187				
10	99.5	189	0.901	1,085	98.5	161	0.871	920	−51	18
		172				95				

* High and low values.

grams in 10 days. Fasting was deemed too drastic a treatment for an adrenalectomized animal; in this respect the experiments herein reported are more physiologic than those previously recorded.

RESULTS. A summary of the individual experiments is given in table 1. The average body weight loss after adrenalectomy was 30 grams. The normal mean values for oxygen consumption agree with the values obtained in a series of determinations on 119 animals kept under the same conditions and previously reported (2, 4). The oxygen consumption of fasting and non-fasting rats under these conditions is the same. Variation between different animals is from 0.150 gram to 0.230 gram O_2 consumption per 100 grams of rat per hour. Individual variations are given in the table.

The caloric output was calculated using the surface area formula of Diack (6). The normal mean number of calories of heat production for 75 determinations on 10 animals in this series is 1068 per sq. m. per 24 hours with a standard deviation of 107 calories. This value compares favorably with that previously reported as 1040 for fasted animals. After adrenalectomy the mean caloric output is 10 per cent lower for the ten animals (93 determinations). However, since the metabolism of individual animals increased for the first few days after adrenalectomy and before death, the average figure does not illustrate the true fall in metabolism. The lowest metabolic values were obtained usually on the eighth day after adrenalectomy and on the day before death.

The respiratory quotient of adrenalectomized animals consuming approximately 6 grams of food per day was found to vary between 0.750 and 0.915. Animals having a low body temperature, one or two days before death frequently exhibited a high R.Q. The high R.Q. indicates a normal carbohydrate metabolism.

The oxygen consumption after adrenalectomy was decreased in all animals except no. 2 (table 1). Even in this animal the total metabolism decreased 3 per cent before death.

DISCUSSION. Table 1 while demonstrating the overall decrease in metabolism does not indicate the marked rise in metabolism that occurred in 8 of the animals within one to four days after adrenalectomy and in all of the animals a few days preceding death. The per cent caloric decrease shown is calculated using the lowest values obtained before death. These low values correspond with the lowest figures for oxygen consumption given in the table.

Changes in metabolism after adrenalectomy in rats associated with changes in body temperature have been observed previously (1). The day to day fluctuation in body temperature observed in normal animals does not correlate with their variations in metabolism. In the adrenalectomized rat in a constant temperature room, a rise or fall in temperature was invariably associated with an increase or decrease in caloric output. The temperature of these animals permitted a good estimate to be made of their metabolism.

CONCLUSIONS

1. After bilateral adrenalectomy in the rat there is a reduction of approximately 25 per cent in the total metabolism.

2. The adrenalectomized rat after consuming carbohydrate food evinces an R.Q. comparable to a normal animal.

3. A rise in body temperature and metabolism was observed in acute adrenal insufficiency in 8 of the 10 animals studied.

4. Normal rats maintained on a restricted diet under similar temperature conditions do not show a reduction in total metabolism.

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GLUTATHIONE IN FROG TISSUES WITH SPECIAL REFERENCE TO PERIPHERAL NERVE[1]

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Received for publication January 27, 1937

When oxygen is admitted to an asphyxiated nerve the oxygen consumption is greater than the normal resting level for a period of an hour or so (Gerard, 1927a; Schmitt, 1929; Fenn, 1930). During this period the respiratory quotient is so low as to indicate that the excess oxygen uptake is concerned with processes other than those of complete oxidation (Schmitt, 1929). It has been suggested (Gerard, 1927b) that the reoxidation of glutathione, which had been reduced during anoxia by activated substrate molecules, might account for this phenomenon. This function as an oxidative reserve would require glutathione to be present normally, at least in part, as disulfide glutathione (GSSG). Determinations on other tissues indicate rather, that it exists chiefly as sulfhydryl glutathione (GSH) (Hopkins and Elliott, 1931; Meldrum and Tarr, 1935; Quensel and Wachholder, 1935). However, Binet and Weller (1936) have recently presented evidence that the GSSG content of some tissues, among them brain, may amount to 25 per cent of the total glutathione (GT) normally present.

No method, with the possible exception of that of Binet and Weller (1936), is available for the determination of small quantities of tissue GSSG. However, a shift in the equilibrium between oxidized and reduced glutathione in the tissue, yielding an increase in the reduced form, should be detectable by determining the GSH concentration before and after the change has taken place. This would be true only on the condition that the increasing GSH did not enter into an accompanying secondary reaction which destroyed, or reconverted it to the oxidized form.

Since frog nerves were used in all previous studies on the oxidative reserve it was necessary for the present work to use the same tissue. Because of the small quantity of GSH in frog nerves a method satisfactory both as to specificity for GSH and adaptability to small amounts of tissue containing low initial concentrations of reduced glutathione had first to be worked out. No quantitative data on GSH in any frog tissue being avail-

[1] The material presented in this paper is part of a thesis submitted to Washington University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

able when this work was in progress, these preliminary experiments on methods were extended to include a variety of tissues beside nerve.

EXPERIMENTAL. The iodometric titration method (Quensel and Wachholder, 1935) and the colorimetric method (Fujita and Iwatake, 1935) proved poorly adaptable to the present problem as well as lacking in specificity for GSH. The manometric method developed by Woodward (1935) satisfies both requirements and was adopted. An extensive survey of several of the currently used methods will be given in another place.

Preparation of tissues. The tissues were obtained from *R. pipiens* or *R. clamitans* from several different shipments. With the exception of one group all were "winter frogs", the animals being kept without feeding in the ranarium until used. Tissues were removed as rapidly as possible after killing, freed from extraneous tissue, rinsed in Ringer solution, the excess liquid removed by gently pressing against filter paper, and dropped into cold weighing vials. During the weighing and until the sulfosalicylic acid was added, the temperature of the tissue was kept below 5°C. Five to 25 minutes per frog, depending on the number of tissues used, were required to remove and chill the tissues.

Preparation of filtrates. Protein free extracts were prepared with 2 per cent sulfosalicylic acid (Quensel and Wachholder, 1935). The best results were obtained by grinding, in an agate mortar to a soft paste, mixtures of tissue, concentrated sulfosalicylic acid, and sand (especially purified to remove traces of heavy metals). This permitted quantitative transfer of the ground tissue into small centrifuge tubes and protected against GSH oxidation during the preparation. The volume was adjusted by addition of water to give a volume of mixture equivalent to 5 cc. per gram of tissue. The contents are thoroughly mixed, centrifuged at high speed, and the supernatant fluid withdrawn for the determination of GSH.

Determination of GSH. The procedure is essentially that of Woodward (1935). The manometer cups with a total volume of about 11 cc. had a single side arm and no alkali inset. Methylglyoxal was prepared[2] by the method of Riley, Morley and Friend (1932) and had a boiling point ranging from 54 to 58°C./50 mm. Dilute solutions were standardized by Friedemann's method (1927). Acetone-yeast was prepared according to the method of Albert, Buchner and Rapp (1902), about 500 grams of baker's yeast being treated at one time.[3]

Most of the GSH is removed from the acetone-yeast by washing with water or dilute potassium chloride. However, mixtures of washed acetone-yeast, sodium bicarbonate, and methylglyoxal produce carbon dioxide at

[2] For the preparation of the methylglyoxal we are indebted to Dr. J. H. Gardner and Mr. J. W. McCrackin of the Department of Chemistry, Washington University.

[3] We wish to thank Dr. Carol Neff of Anheuser-Busch Inc., St. Louis, who kindly supplied us with fresh starch-free baker's yeast directly from their yeast plant.

a constant rate during the course of the experiment. Side reactions in the yeast and between yeast and methylglyoxal contribute to this blank. Experimental values for carbon dioxide production due to added GSH must be corrected by subtraction of a comparable blank to obtain true extra carbon dioxide production. Although comparable blanks are approximately the same, variations sometimes occur of the same order of magnitude as the extra carbon dioxide production given by 0.005 mgm. GSH. With standard GSH solutions the difficulty may be avoided by determining the average carbon dioxide production per unit time for a number of comparable blanks, which is used as the correction factor for the average carbon dioxide production per unit time from a comparable series with a given GSH concentration. An example of a standard curve obtained by this means from a small number of comparable determinations (from 5 to 15 per GSH concentration) is shown in figure 1.

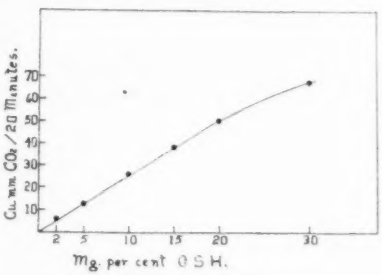

Fig. 1. Standard GSH curve (100 mgm. yeast)

If the source, quantity used, and treatment of the acetone-yeast is kept the same in the determination of GSH with standard solutions and tissue filtrates, it is not necessary to repeat the standard curve with each experiment. Limitation of the amount of available extract, especially with peripheral nerve, prevented strict application of the above procedure in the determination of the extra carbon dioxide production with tissue filtrates. Wherever possible the average of duplicate blanks determined in the same run as the experimental values was used as the correction factor. In other cases the value of the single blank was used. The consistency of the results where duplicate determinations were made with the same filtrate justifies this procedure. For each determination 0.25 cc. of standard or unknown solution (before neutralization) was placed in the side arm, thus permitting direct comparison between extra carbon dioxide production of unknown and standard solutions.

Nerve asphyxiation. Thunberg tubes with one side arm were used in the experiments with vacuum asphyxiation. The nerves were placed in the main chamber in 0.25 cc. of Ringer solution and the calculated amount of concentrated sulfosalicylic acid placed in the side arm. After evacuation of the experimental tube, both tubes were closed and immersed in a thermostat at 25°C. and gently shaken. At the end of the asphyxial period the concentrated acid was tipped onto the nerves, the seal broken and the nerves and fluid removed. With mixtures of carbon monoxide and approximately 2 per cent oxygen asphyxiation was produced in a special

chamber (Schmitt and Cori, 1933). When the treated nerves became non-irritable (spike height of the action potential, determined oscillographically, drops to zero) the nerves were removed and plunged into sulfosalicylic acid. As this required less than ten seconds appreciable recovery from anoxia was prevented. Control nerves were treated similarly except that the chamber was filled with air.

RESULTS. *GSH in frog tissues.* The GSH determinations for a few representative tissues of the frog are listed in table 1.

Individual variations were reduced by the use of 5 to 12 frogs for each experiment. The experiments are not strictly comparable because of the necessity of using frogs from different shipments. Thus the species and previous treatment of the groups varied. In 2 of the 11 experiments with kidney tissue no GSH was found and the filtrates of the other tissues from the same animals gave the minimum values listed. Discrepancy in the

TABLE 1

Glutathione content of normal frog tissues

All results are expressed as milligrams per 100 grams of tissue

TISSUE USED	NUMBER OF EXPERIMENTS	GLUTATHIONE CONCENTRATION		
		Maximum	Minimum	Average
		mgm.	*mgm.*	*mgm.*
Brain	11	60	21	35
Heart	7	46	17	32
Kidney	11	67	0	29
Nerve	6	15	5	10
Muscle	2	7	6	6

"blank" may account for the apparent complete absence of GSH in these two cases. Where the species and previous treatment were the same, or nearly so, better agreement was found. For example, in 4 different experiments on the same shipment of *R. clamitans,* using 12 animals in each, the values for brain were 36, 31, 41, and 44 mgm. per cent. In addition to the data in table 1 three single experiments on blood, stomach muscle, and spinal cord gave GSH values of 3, 22, and 22 mgm. per cent respectively.

These results support the suggestion (Geiger, 1935; Ashford, 1933) that tissues metabolizing mainly glucose have a higher concentration of GSH than a tissue such as skeletal muscle where GSH is not needed for carbohydrate metabolism.

Glutathione in anaerobic nerve function. Using the most conservative value for the oxygen debt of nerve (2.7 $mm.^3$/gm./ hr. of anoxia, Schmitt, 1929), the calculated increase in GSH after 4 hours of asphyxia, if all the

extra oxygen was used for restoration of GSSG, is 56 mgm. per cent. This is an increase of more than 4 times the average GSH content of normal nerve. In addition it should be emphasized that an increase of this magnitude would raise the GSH value into the range where the determination is considerably more reliable. Variations in the "blank" could not mask an increase from 15 mgm. per cent in the normal nerve to 60 mgm. per cent following asphyxia.

The results presented in table 2 show no increase in the GSH content of experimental over control nerves. Moreover, the absolute GSH concentration in all the experiments is the same within the experimental error of the method, despite the fact that each group of nerves received different

TABLE 2

Glutathione content of frog peripheral nerve after asphyxia

All results are expressed as milligrams per 100 grams of tissue

EPPERIMENT NUMBER	NUMBER NERVES USED	TREATMENT	DURATION OF EXPERIMENT	GLUTATHIONE CONCENTRATION
			hrs.	*mgm.*
F9-a	8	None		10
F9-b	8	Vacuum, in cold	4	10 (10, 10)*
F9-c	8	In air, in cold	4	10 (10, 10)
F12-a	12	Vacuum, 25°C.	8	14 (7, 20)
F12-b	12	In air, 25°C.	8	18 (16, 21)
F14-a	12	Vacuum, 25°C.	10	13 (15, 11)
F14-b	12	In air, 25°C.	10	15 (12, 17)
F22-a	9	$CO—O_2$, 25°C.	3.5	15 (15, 15)
F22-b	9	In air, 25°C.	3.5	16 (15, 17)

* The figures in parenthesis represent the values obtained in duplicate determinations on the same filtrate.

treatment. It must be concluded, therefore, that GSSG is not the oxidative reserve of frog peripheral nerve. Brachet (1934) similarly concluded that glutathione plays no rôle as an oxidative reserve in frog eggs. Borger, Peters and Kurz (1933) found no increase in the GSH in a wide variety of tissues kept for long periods in the absence of oxygen. This was confirmed by Lang (1935).

Discussion. The present experiments have by no means excluded the possibility that disulfide or sulfhydryl groups may play an important rôle in nerve function. The consistency of the values obtained under varying conditions suggests the possibility that GSH may be concerned in some cyclic mechanism which tends to maintain the absolute amount of GSH

essentially constant.[4] Schmitt and Skow (1935) report greater loss of GSH when added to the medium during studies on nerve respiration, than can be accounted for by autoxidation. It is possible, therefore, that GSH is able to react with other constituents in the nerve. A shift in the oxidation-reduction equilibria of the nerve during anoxia might induce reduction of the bound –SS– groups of the nerve proteins, producing in turn GSSG to react with additional activated substrate. Mirsky and Anson (1934, 1936) present evidence that cysteine is capable of reducing bound –SS– groups in natural proteins during relatively short periods of anoxia, and GSH may act in the same manner. Hopkins and Elliot (1931), working with pure glutathione, confirmed earlier observations of Hopkins that tissue is able to oxidize GSH in the complete absence of oxygen. Waldschmitz-Leitz (1931) advanced such a view to explain the activating effect of GSH on autolysis. Lang (1935) could demonstrate no increase in GSH during autolysis, and indeed none need necessarily be expected. The increase in GSSG which he reports after long periods of decreased oxygen supply might be expected after the available supply of activated substrate had diminished.

Although no quantitative data are available on the bound -SS- groups of nerve proteins, one can easily demonstrate an intense nitroprusside reaction in the residue of nerves after precipitation with sulfosalicylic acid. The possibility is still open therefore, that disulfide and sulfhydryl compounds may play an important rôle in the oxidative processes of nerve during anoxia even though no gross changes are detectable in the GSH concentration.

The author is indebted to Dr. Francis O. Schmitt, under whose direction this investigation was carried out, for constant assistance and advice.

SUMMARY

1. The manometric method for the quantitative estimation of GSH has been successfully applied to frog tissues where the quantity of available tissue is limited and the initial GSH concentration is low.

2. The GSH concentration in several tissues of the frog (*R. pipiens* and *R. clamitans*) are given.

3. The system GSSG $\rightleftharpoons$ GSH does not function as the oxidative reserve in peripheral nerve. No change in the GSH content occurs during anoxia under various conditions.

4. The results do not exclude the possibility that GSH functions in some cyclic mechanism or that disulfide and sulfhydryl groups in the nerve may play an important rôle in the oxidative mechanism of nerve.

[4] It is of interest in this connection to note an experiment in which a group of nerves was stimulated continuously in air at frequency of sixty maximal shocks per second for 10 hours; their GSH content was determined immediately thereafter and compared to that of an unstimulated control group. Duplicate determinations with both filtrates gave a GSH value of 15 mgm. per cent in both cases.

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THE PTYALIN CONTENT OF HUMAN SALIVA IN OLD AGE[1]

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Received for publication January 28, 1937

It is known that the saliva of infants has less ptyalin than that of adults (1, 2). There is apparently no available literature on the salivary secretion of old people, and therefore the work presented here was undertaken.

The ptyalin content of saliva was tested by the method described by Hawk and Bergeim (3). One cubic centimeter of saliva was diluted to 100 cc. with distilled water. Five cubic centimeters of a two per cent solution of soluble starch, 2 cc. of a 1 per cent NaCl solution, and 2 cc. of a phosphate buffer solution of pH 6.6 were pipetted into a test tube which was kept at 38°C. Two cubic centimeters of a light yellow iodine solution was pipetted into each of ten small tubes. One cubic centimeter of diluted saliva was added to the starch mixture. At the end of each minute of digestion, two drops of the starch mixture were removed and added to one of the tubes with iodine solution. The time when no change in color appeared in the iodine solution was considered as the end point. If the digestion time was less than 5 or more than 20 minutes a different dilution of saliva was used, such as would give a digestion time of about 10 minutes. One unit of ptyalin is expressed as the amount required to digest 5 cc. of a 1 per cent soluble starch solution to the achromic point in 10 minutes. The number of units of ptyalin in 1 cc. of the saliva tested equals 10 over the number of minutes to the achromic point times the dilution of the saliva. The saliva was not filtered because filtration reduces its ptyalin concentration. All tests were done in duplicate.

The subjects had no breakfast. They rinsed their mouth with water and the first samples of saliva were discarded. The following samples were put on ice immediately. In a number of persons the effect of stimulation of salivation on ptyalin secretion was tried; the subjects chewed diabetic gum for 30 minutes, expectorating all saliva during that period. They had been instructed not to swallow during the time of chewing. Some of the old patients were not able to chew and expectorate saliva for the full

[1] Aided by the Oscar Aberle Fund. The complete tables of this paper can be obtained from Dr. H. Necheles, Department of Gastro-Intestinal Research, Michael Reese Hospital.

period of 30 minutes because of dryness of the mouth. In these cases the collection of saliva was stopped as soon as the size of the individual samples dropped to practically nothing. A water extract of diabetic gum did not affect digestion time of starch by a given sample of saliva.

A group of 27 aged men and women, ranging between 69 and 100 years, with an average age of 81 years, represents the experimental group. The old people were from a Home for the Aged[2] and a number of them were suffering from chronic ailments and debilities of old age. It is believed that the various ailments or dental plates did not influence the secretion of salivary ptyalin. A group of twelve young individuals with an average age of 25 (21–31) years, was used as controls.

RESULTS. *Ptyalin content of basal salivary secretion.* Salivary ptyalin in the group of younger subjects had a concentration of from 2.4 to 22.2 units with an average of 10.15. The group of aged subjects had a ptyalin concentration of from 0.19 to 0.48 with an average of 0.303. The variation in the second group was much less than in the first group. The average of 0.303 of the older group is only one thirty-fourth of that of the younger group. It may be of interest to point to the absence of potassium thiocyanate in the saliva of 5 out of 16 subjects in the second group (i.e., 31 per cent).

Ptyalin content of appetite salivary secretion. When salivary secretion was stimulated by chewing gum, the same fundamental difference in salivary ptyalin content was found in the two groups. The volume of saliva in the younger group varied between 13.6 and 15 cc. with an average of 14.2 cc., while that of the older group varied between 1.5 and 15 cc. with an average of 5.8 cc. The ptyalin content of the saliva in the first group varied from 2 to 14.3 units with an average of 8.2, while that of the second group was found to vary between 0.18 and 0.42, with an average of 0.28. The specific gravity of the saliva of old people was higher than that of the younger group (1.009 against 1.004). Non-protein nitrogen, total nitrogen and urea were estimated but no value is attached to these data in connection with ptyalin secretion. Potassium thiocyanate was present in all samples of the older group, including one person who had none in his basal secretion. One subject of the younger group, a non-smoker, had no potassium thiocyanate in his saliva obtained after chewing. In the older group the ptyalin concentration in basal and stimulated salivary secretion did not vary much with exception of two subjects, while in the younger group greater variations were noted. A repetition (on different days) of appetite stimulation of salivation evidenced remarkable constancy of ptyalin secretion in the older group. The basal secretion of ptyalin in young and old persons was found to be rather constant on different days (always

[2] We acknowledge the coöperation and help of Doctor Singer, attending physician, and of Miss Fadden, attending nurse, of the Home for the Aged.

taken at the same hour of the morning). In three of the older subjects the volumes of secretion varied greatly, but ptyalin concentration, as well as specific gravity and concentration of potassium thiocyanate remained constant.

Discussion. Chittenden and Cannon showed that salivary digestion may continue effectively in the stomach, and Bergeim (4) found that within 15 to 20 minutes, 76 per cent of a meal of potatoes may be transformed to maltose in the normal human stomach.

All mechanisms of the body have a wide range of safety. Pancreatic ferments are sufficient to digest all the starch ingested, but in the case of old people the burden of starch digestion is thrown on the pancreas. Nothing is known about pancreatic secretion of amylase in the aged, and little about over-taxation of the pancreas by carbohydrates. As to the causation of such deficiency little could be found in the literature. Cachexia (5–7) was present in only a few of our aged subjects. A. J. Carlson and collaborators (8) found that more ptyalin was secreted with the faster rates of salivary secretion, and while the rate of stimulated secretion was rather small in old people, their basal secretion had a very low ptyalin content already. A possible cause seems to lie in the atrophy of age of the ptyalin secreting gland cells.

SUMMARY

The resting and stimulated salivary secretion of ptyalin has been estimated in a group of 12 subjects with an average age of 25 years and a group of 27 subjects with an average age of 81 years. The "basal" saliva of the older group contained 0.303 unit of ptyalin per cubic centimeter of saliva, while that of the younger group contained 10.15 units. When salivary secretion was stimulated 0.28 and 8.2 units respectively were found. Therefore, old people have a deficiency of starch digestion in the mouth and stomach, and nearly the entire weight of carbohydrate digestion is thrown on the pancreas; while in the mouth and stomach of young people 50 grams of white bread will be digested easily, only 1 per cent of it will be digested by old people. The implications of these findings are discussed.

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ASPECTS OF SPLANCHNOMEGALY ASSOCIATED WITH THE ACTION OF PROLACTIN[1]

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Received for publication January 28, 1937

During recent years, in both clinical and experimental research, splanchnomegaly has been rather generally regarded as a result of the action of the growth hormone. The evidence that the condition is traceable to the anterior pituitary is nearly conclusive; and current conceptions of the nature and rôle of a "growth hormone" have permitted practically no other interpretation of a generalized overgrowth of the viscera. It is certain that the A.P. produces three or four hormones having indispensable and extensive action upon and through other endocrine glands; but we believe that the existence of a hormone which does not act through such glands—and does act directly upon cells as a growth stimulant—has not been proved. The data reported here indicate that in doves and pigeons splanchnomegaly is rather closely associated with action (primary or secondary) of the anterior pituitary hormone, prolactin. The present contribution, like three or four others that have preceded it (1, 2, 3), tends to allocate aspects of body growth to other pituitary hormones and thus to question the separateness and singleness of a "growth" factor in the pituitary.

The rôle of specific and individual difference in animals in aspects of either body growth or of splanchnomegaly is of first importance—even though the basis of such difference is unknown or very little known. To most of the quite impure extracts that have been called "growth hormone" rats and dogs have reacted with particularly good growth, while some other species or individuals have shown much less growth. We have elsewhere noted that our prolactin preparations tend to give good body growth in pigeons (4), and but little in dwarf mice (2); while thyrotropic hormone decreases body weight in pigeons and increases it in dwarf mice (2). We have thus been led to the view that different species, and probably also different individuals within the species, vary as to the contribution of the pituitary which they require for an extension or prolongation of their body growth. That view is applicable to the data for splanchnomegaly and

[1] Aided by a grant from the Carnegie Corporation of New York.

body growth presented here. With prolactin we have partly failed to get these responses in the rat; we have obtained them in doves and pigeons.

RESULTS. *The action of prolactin in the hypophysectomized pigeon.* It was earlier shown that when the B.M.R. is measured at the bird's critical temperature prolactin markedly increases (33 per cent) the heat production of hypophysectomized pigeons (5). In another study this action of prolactin on the B.M.R. was found (and since confirmed, unpublished) also in thyroidectomized birds (3); and a synergism of prolactin and thyrotropic hormone on the B.M.R. of normal pigeons was likewise found in that study. This clearly indicated a widespread or generalized action

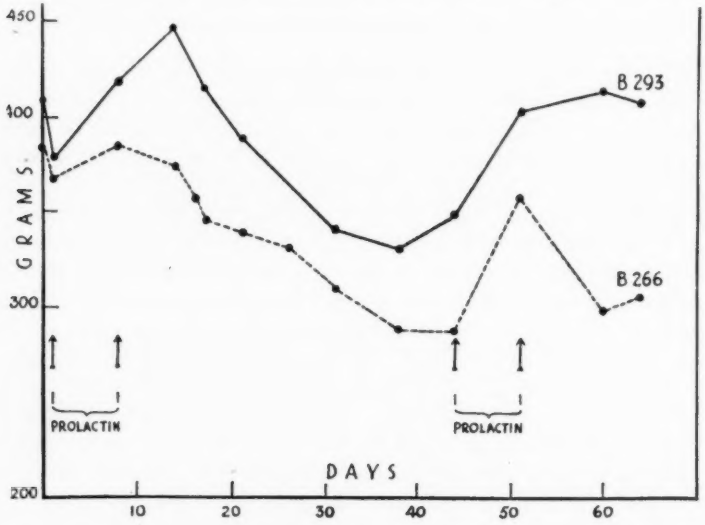

Fig. 1. Showing that prolactin (495 H) maintains, during first 10 days, and later increases body weight in two completely hypophysectomized pigeons.

(direct or indirect) of this hormone in the body. Figure 1 shows that in two hypophysectomized pigeons prolactin was able to prevent the pronounced decrease in body weight which always immediately follows hypophysectomy, and to restore the normal body weight after full effects of the operation had been permitted to develop. This restoration of the body weight to or above normal has been repeated on many hypophysectomized pigeons; this result is obtained in practically all cases and with prolactin previously heated to 60°C. for 5 hours.

The liver of the hypophysectomized pigeon responds to prolactin administration by very marked enlargement. In 10 uninjected controls an average liver weight of 5.3 grams was found; in 10 comparable birds in-

jected with prolactin from 4 to 10 days an average of 14.4 grams was found. The highest uninjected value was 7.69 grams and the lowest treated value, 8.60 grams.

TABLE 1

Aspects of splanchnomegaly associated with the phase of rapid growth (1.3 months) in uninjected young White Carneau pigeons and in similar young injected for 4 days with prolactin

The standard deviation of the mean is given with each average value

ROUTE OF INJECTION	NUMBER OF BIRDS	AGE	DAILY DOSE	BODY WEIGHT	LIVER	INTESTINE			THYROIDS	CROP-GLANDS
						Length (gizzard to anus)	Full weight	Empty weight		
		mo.	*mgm.*	*grams*	*grams*	*cm.*	*grams*	*grams*	*mgm.*	*mgm.*
Uninjected; youngest (1.3 mo.) group in extremely rapid growth										
	12	1.3		298± 4.9	9.3±0.3	117.8±2.2	16.0±0.5	10.3±0.4	30.5±1.7	1670± 70
	15	1.5		457±11.6	10.0±0.3	117.8±1.4	17.7±0.6	13.2±0.4	33.3±1.5	1490± 49
	36	1.5		431± 5.4	9.8±0.3	115.9±1.7	15.4±0 3	10.5±0 2	32 4±1 0	1630± 53
	27	2 2		461±10.7	10.7±0.4	110.8±2.0	19.1±0.6	12.0±0.3	41 1±3 1	
Control	30	2.2		476± 8.6	9.7±0.4	104.0±2.4			39.6±1.9	1245± 33
Injected with iso-soluble fraction no. 474 = F.S.H. + thyrotropic										
Two routes*	10	2.2	5	453−35†	8.4±0.6	102.3±3.4	15 8±0 9		77.7±12.0	1150± 64
Injected with iso-insoluble fraction no. 469 = prolactin										
Intravenous	10	2.2	1	498+15	10.7±0.5	113.1±3.4	21.7±2.0		42.7±2.4	1610±113
Intraperitoneal	10	2.2	1	465+34	11.5±0.8	115.5±2.7	22.0±3.0		39.0±2 0	1890± 91
Intramuscular	15	2.2	1	457+24	10.8±0.6	111.1±2.9	18.2±1.0		40.6±1.8	2355±188
	10	2.2	1‡	448+30	11.8±0.6	109.5±2.3	17.3±0.7		36.9±1.7	4170±170
Intracutaneous	10	2.2	1	460+43	13.3±0.9	117.3±4.6	20.5±1.5		40.5±1 8	3790±233
Subcutaneous	10	2.2	0.1	466+ 5	9.3±0.7	104.4±1.9	17.1±0.6		33.1±2 0	1255± 59
Subcutaneous	10	2.2	0.3	460+19	10.0±0.4	109.2±2.7	16.7±1.0		37.4±2.4	1935±238
Subcutaneous	15	2.2	1	459+47	12.6±0.6	125.4±4.1	20.5±1.2		37.4±2.5	3940±232
Subcutaneous	10	2.2	1‡	459+16	13.2±0.8	117.0±3.1	20.4±1.2		36.9±1.3	4580±385
Subcutaneous	10	2.2	5	453+75	16.4±0.9	127.0±4.4	27.9±1.7		42.0±3.6	5845±476
Subcutaneous	9	2.2	10§	487− 7‖	14.7±0.8	117.3±4.1	23.5±1.7	15.3±0.8	47.9±6.1□	5070±376

* Intramuscular (5) and subcutaneous (5).
† Gain or loss of weight is indicated for all injected birds.
‡ Divided into equal thrice daily injections.
§ No. 495 H2 = heated prolactin used.
‖ Four birds each lost over 30 grams (2 others lost less) due to acute infection.
□ One extreme thyroid weight alone changed this average from 40.0 mgm.

Phenomena associated with normal but extremely rapid growth. From data for pigeons supplied by this laboratory Brody (6) found that during their first two or three weeks of life pigeons show a higher relative rate of body growth than does any other laboratory animal. The data at the top of table 1 should be examined with an appreciation of this speed of growth

in mind. The youngest group (1.3 mo.)—39 days from egg laid, or 21 days from hatching—has increased its hatching weight of 18 grams to 298 grams; within the next 6 days this weight would further increase to more than 430 grams, or more than 44 per cent. It will be seen that the livers of the youngest group were only 10 per cent smaller than those of 2.2 month birds whose body weights were 57 per cent larger; and that in birds only 6 days older liver size was essentially equal to that found in birds of 2.2 months. The intestines of these birds at three weeks after hatching were actually longer than those of wholly similar birds 27 days older (2.2 mo.) and 57 per cent heavier. The full weight and the empty weight of the intestines of this youngest and rapidly growing group was but slightly less than corresponding weights of the oldest groups in which growth was practically completed. The thyroid glands have not completed their growth at 1.3 months.

The crop-glands of the birds aged 1.3 and 1.5 months provide a very significant item of information. These glands, and more particularly those of the 1.3 month group, were found to be *absolutely larger* (34 per cent) than those of the much heavier 2.2 month birds; and their macroscopic and microscopic study *shows that they were giving* the normal prolactin response (a thickened proliferating mucosa). Since we have earlier found that prolactin given by mouth will not enlarge the crop-glands of pigeons of a slightly older age it seems that the source of the prolactin which is enlarging these glands must be sought in the growing squab's own pituitary. Also, since, as shown in the lower part of table 1, prolactin administered to similar birds aged 2.2 months (or to hypophysectomized pigeons) leads to *a*, resumption of body growth; *b*, still larger livers; *c*, longer and heavier intestines—these aspects of splanchnomegaly and growth are effects associated with prolactin. Hypophysectomy in the pigeon at 2 weeks after hatching quickly stops its growth (unpublished data).

Data for weight of pancreas in these rapidly growing squabs could not be included in the table. They are as follows for the various age-groups: 1.3 months, 1.48 $\pm$ 0.04; 1.5 months (two), 1.83 $\pm$ 0.11 and 1.61 $\pm$ 0.05; 2.2 months, 1.32 $\pm$ 0.09. The standard deviations of the mean, together with the lower body weights of the younger groups, make it highly probable that the pancreas also shares in this visceral overgrowth in squabs at three and four weeks after hatching.

Results from injection of an isosoluble pituitary preparation. Birds of this same race (like many other racial groups studied by us) injected for 4 days with an isosoluble fraction of beef anterior pituitary gave the following comparisons with their uninjected control: Loss of body weight; smaller liver weight; intestinal length and weight unchanged or smaller; gonads enlarged (not shown here); thyroids enlarged; and crop-glands unchanged. The isosoluble fraction used was free from intermedin and pos-

terior lobe hormones, and should probably contain easily detectable amounts of all A.P. hormones *except prolactin*.

Results with prolactin. Other similar birds were injected with prolactin. There is no possibility that this isoinsoluble preparation was contaminated with intermedin or posterior lobe hormone, and tests showed it to be essentially free from F.S.H. and thyrotropic hormone. It has earlier been pointed out that when prolactin is injected by various routes, a highly variable response of the crop-glands is obtained (7), and that the intracutaneous and subcutaneous routes give the highest response in the crop-glands. The tabulated data show that these same routes (with comparable dosage) give also largest livers and longest intestines. Higher dosage (and dividing the daily dose) increased these effects. Thus prolactin treatment for 4 days induced body growth together with prompt and disproportionate enlargement of liver, intestines and crop-glands.

In connection with another study (4) published alongside this one we have shown that also in normal adult doves and pigeons of various races prolactin induces body growth and marked overgrowth of the liver. The nature of the induced hepatic changes in both young and mature is under study and will be separately reported.

Discussion. The present results extend, confirm and clarify, and are in turn supported by, data previously published from this laboratory. Riddle and Flemion (8) were first to call attention to the enlargement of the liver by something of anterior pituitary origin. It was later found (9) that the body weight of doves and pigeons cyclically increase by about 8 per cent during the period of incubation and this was "believed to have a hormonal basis which in time relations at least is associated with the release of prolactin." Another study (10) showed that at 10 to 25 days after hatching the young pigeon has a very much higher B.M.R. than at any later stage of life. Again, a synergizing action of prolactin on thyrotropic hormone for growth-promotion in the dwarf mouse was reported (2). These several findings are obviously related to the rôle of prolactin in body growth and in overgrowth of the viscera. The relatively very high prolactin content of the pituitaries of cattle embryos (11) also takes its place in this same picture. Prolactin is associated with early and rapid growth—and with resulting physiological overgrowth of viscera in some species in which overgrowths of both body and viscera can later be induced by prolactin administration. Best and Campbell (12) got marked liver enlargement in rats treated with an anterior pituitary extract that probably contained all pituitary hormones.

Some years ago two of us (13) inclined to regard the enlargement of the liver following the administration of the pituitary preparations then available as "a specific response to no pituitary hormone but as a non-physio-

logical (pathological) response to unusual quantities of two or more potent hormones which never normally thus coexist in the blood." This view was much influenced by the current acceptance of growth hormone as a separate and single entity. Moreover, some of the "growth" preparations then tested by us produced marked liver enlargement although their prolactin content was certainly slight. Unless those preparations, which were sent to us by others, contained a preservative which affected liver size we are unable to explain those results. Again, we obtain neither increase in body size nor liver enlargement in a very small proportion of the many hundreds of doves and pigeons injected with prolactin; but we presume that individual difference, refusals to take food and rare acute infections (such as occurred among the group listed at bottom of table 1) account for these highly exceptional cases.

SUMMARY

Body growth, with rapid and excessive increase of liver weight, is obtained in completely hypophysectomized pigeons by the administration of relatively highly purified preparations of prolactin.

At three weeks after hatching the rapidly growing young pigeon shows crop-gland stimulation from prolactin produced in its own pituitary. At this stage it shows, in addition to an amazing rate of body growth, relative overgrowth in liver, intestines, and probably in the pancreas.

When these birds are somewhat older, and in growth stasis, the administration of prolactin is followed by a resumption of body growth and by overgrowth of liver and intestines. Administration of an isosoluble pituitary preparation, known to be rich in F.S.H. and thyrotropic hormones, and probably containing all anterior lobe hormones except prolactin, produces none of these effects.

Though these aspects of splanchnomegaly have not been induced in the rat by the same preparations of prolactin which regularly produce them in the dove and pigeon positive results are regularly obtained in the latter species. The allocation to prolactin of these aspects of growth and overgrowth in these species provides a basis for further doubt as to the separateness or singleness of a "growth" hormone in the pituitary.

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THE MECHANISM OF THE ANTI-GONAD ACTION OF PROLACTIN IN ADULT PIGEONS[1]

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Received for publication January 28, 1937

We have earlier reported that prolactin shows a pronounced anti-gonad action in mature pigeons, though this action is not obtained in very immature birds (1, 2). That both the immature (3, 4) and the mature (1, 2, 5) gonads of these birds are stimulated to rapid growth by gonad-stimulating hormone has also been reported. The present study is an investigation of quantitative relationships existing between these two hormones in their opposed action on mature gonads. The results seem to clarify the mechanism of the anti-gonad action of prolactin.

Many data bearing on this subject, but not readily presented here, were obtained during the past four years incidental to other studies. It is found convenient, and important to other current publication, to tabulate here the changes in body weight and liver weight which occurred in the birds used in this study.

Data obtained from a group of adult male ring doves are first considered. Table 1 shows the effect of prolactin injected alone, of an F.S.H.$^+$ fraction injected alone, and of different amounts of the latter injected with a standard amount (50 units; 100 in one case) of prolactin. The prolactin preparation (no. 380) used was quite free from F.S.H. as shown by tests on the immature dove testis. The F.S.H.$^+$ preparation (no. 198) was an isosoluble fraction, practically free from prolactin; it was shown by tests on immature dove testes and rabbit ovulation tests to be rich in F.S.H., and by dove thyroid and B.M.R. tests on pigeons to contain fair amounts of thyrotropic hormone.

With doses of 50 units of prolactin alone testis weight was reduced about 85 per cent, which is approximately the extent of their reduction after complete hypophysectomy (87 per cent in 11 pigeons after 10 days). With the addition of 0.05 mgm. F.S.H.$^+$ this reduction was about 55 per cent; with 0.1 mgm. about 6 per cent; with 0.1 mgm. and 100 units prolactin, about 15 per cent; with the standard 50 units prolactin and 0.2 mgm. F.S.H.$^+$ an increase of testis weight of about 20 per cent is indicated. It

[1] Aided by a grant from the Carnegie Corporation of New York.

is evident therefore that 0.1 mgm. of this highly potent F.S.H. preparation was the amount required to neutralize the adverse action of 50 units of prolactin on the adult testis; 0.05 mgm. did not neutralize the prolactin effect; 0.2 mgm. wholly overcame the adverse effect of prolactin and induced additional testis growth (about 20 per cent). When no prolactin was given 0.1 mgm. F.S.H. gave an additional testis growth of about 23 per cent.

These data clearly show that the anti-gonad action of prolactin is not exerted directly upon the testicular tissue. Prolactin depresses testis size only when the available F.S.H. is below a certain value—a value which in all probability corresponds closely with that which normally sustains or maintains (large) testis size. Other data have shown that the pituitary of the very immature pigeon has no F.S.H. to release (6) and in such birds the high sensitivity of their minute testes to injected F.S.H.—even in the presence of prolactin and all other A.P. hormones—is well established; that fact is inconsistent with a direct antagonistic action of the two hormones on gonadal tissue. These results, supported by those obtained in two other series of tests described below, therefore indicate that prolactin stops the F.S.H. output of the dove's own pituitary. From this it follows that we have obtained also a measure of the bird's daily pituitary output of F.S.H.—the amount required to maintain the adult testes of ring doves. This value is found to be approximately equal to 0.1 mgm. of preparation no. 198; and this quantity of this preparation was shown (in other tests) to be precisely the dose required to cause ovulation in the 4-month New Zealand rabbit. The daily output of F.S.H. by the adult male dove's anterior pituitary is thus indicated to be one M.O.D. in the rabbit. Of course there are possibilities of relative inefficiency of twice daily injections (though in doves and pigeons we find no plain difference between once daily and thrice daily injections of F.S.H.) and of more rapid destruction of injected hormone than of physiologically released hormone.

Another point of consequence is shown by these data. Since F.S.H. and prolactin are not directly antagonistic in their action on the testis it is evident from the data of table 1 that 50 units (10 mgm.) of prolactin no. 380 must have contained much less than the equivalent of 0.05 mgm. no. 198, i.e., much less than one part in 200 of isosoluble material. We are able to show what at first seems to be an antagonism, and finally to show that the apparent result rests upon the suppression of F.S.H. output from the bird's own pituitary, only because the prolactin used was so nearly free from F.S.H.

The isosoluble preparation (no. 198) tended to decrease body weight (initially about 150–170 gm. in all ring doves); it did not increase either liver size or crop-gland weights. Prolactin tended to increase body weight, this increase being greatest with highest dosage and when unopposed by the isosoluble fraction; it caused great increase in liver weight (100 per cent

or more in all groups); and, when difference in sensitivity of the various races is considered, it enlarged the crop-glands essentially in proportion to dosage.

It is widely recognized that adequate short term injections of estrone, progesterone and testosterone stop the output of F.S.H. from the pituitaries of some mammals. A second series of adult male doves were injected with these hormones and with high and low dosage of prolactin for comparison. The results are given in table 2. It will be seen that the two types of dosage—sex hormones and prolactin—affect the testes similarly; and that when both estrone and prolactin were given together (in moderate or low dosage) they appear to reinforce each other's action in reducing testis size. The sex hormones increased neither body weight (normally about 160 grams) nor liver size; prolactin gave marked increases in both.

TABLE 1

Effect of separate and combined administration (injected 10 days, twice daily) of two different A.P. fractions in adult male ring doves

NUMBER OF BIRDS	DAILY DOSE		AVERAGE WEIGHTS OBTAINED				
	Prolactin No. 380	F.S.H. + No. 198	Change in body weight	Liver	Crop-glands	Testes	
						Before*	After
	units	*mgm.*	*gm.*	*gm.*	*mgm.*	*mgm.*	*mgm.*
Normals				2–4	300–400		
3		0.10	−5	2.5	340	930*	1,140
2	50		+16	7.9	5,200	1,300*	183
2	50	0.05	−1	7.1	4,320	1,000*	445
3	50	0.10	+10	7.4	3,440	800*	748
3	100	0.10	+27	6.9	5,050	830*	702
2	50	0.20	−1	9.6	4,610	1,000*	1,205

* Testis weights estimated at biopsy.

Table 3 shows data obtained for effects of separate and combined administration of prolactin and F.S.H. on adult ovaries of common pigeons. In this case the gonad effect is better measured by the number of "yellow ova" produced than by increased weight. The great weight increase of adult bird ovaries during F.S.H. administration is due chiefly to yolk laid down in the few or many ova which pass from the slow-growing white yolk stage to the later rapidly growing stage (after attaining about 5 mm. diameter) in which yellow yolk is formed. In general, the data show that graduated quantities of F.S.H.+ yield graduated increases in the number of individual ova that have assumed the rapidly growing (yellow yolk) stage. Three of the 5 groups of prolactin treated females received that hormone in moderate or small dosage only; it is obvious, however, that only when the concurrent dosage with F.S.H. was very small (0.0, 0.2 or 0.3 mgm.)

was the F.S.H. action measurably opposed by prolactin. Still other tests on pigeons, doves and fowls have shown that under moderate or heavy

TABLE 2

Effect of sex hormones and of prolactin on testes, liver and body weight of adult male ring doves

PREPARATION INJECTED	DOSAGE		NUMBER OF BIRDS	AVERAGE WEIGHTS OBTAINED		
	Quantity daily	Days		Change in body weight	Liver	Testes
Muscle extract, control	5 mgm.	7	10	−5	3.5	1,310
Prolactin*	40 B.U.	13	3	+37	8.9	104
Androsterone (Erugon)*	0.3 cc.	10	2	−2	5.6	230
Progesterone (Proluton)*	0.25 U.†	10	4	−2	2.6	279
Estrone (Amniotin)*	40 R.U.	10	4	−3	2.6	213
Estrone (Amniotin)* and Prolactin	40 R.U. 15 B.U.	10	5	+15	6.9	105
Prolactin	15 B.U.‡	7	4	+13	4.7	510‡

* Injected twice daily.

† Clauberg rabbit unit; probably higher dosage should have been used.

‡ In other groups of doves of this series prolongation of this dosage to 14, 21 and 28 days did not further reduce testis size.

TABLE 3

Effects of separate and combined administration (twice daily injections) of two different A.P. fractions in adult female pigeons

NUMBER OF BIRDS	DAILY DOSE		DAYS INJECTED	AVERAGES OBTAINED		
	Prolactin	F.S.H. + No. 198		Body weight	Liver weight*	Yellow ova
	units	*mgm.*		*gm.*	*gm.*	*number*
Normals					2–3*	0–3
3	60		6	413+33†	3.4	0
5		5.0	6	435+10	2.1	26
3		2.5	6	447+ 5	2.3	14
4		1.2	6	354+12	2.5	13.5
2		0.6	6	465−21	2.2	15
3		0.2	6	284−16	2.9	6
3	15	2.5	6	350+18	3.2	13
3	15	0.6	6	343+ 6	3.0	17
3	60	0.3	6	385+25	3.7	12
3	15	0.2	6	344+37	3.8	3

* Per 100 grams body weight.

† Change in body weight during injection.

dosage of prolactin alone no yellow yolk can form and those present may be resorbed. Since only 0.2 mgm. daily of no. 198 for 6 days was here required

to give a quite definite response it is clear that this type of response in the adult ovary is more readily obtained and measured than is ovarian growth in the very immature ovary (3, 4). Again, the suppression by prolactin of output of pituitary F.S.H. satisfactorily explains the mechanism by which prolactin suppresses estrous cycles in mice (7) and rats (8).

In these tests the 17 birds injected with the isosoluble preparation alone increased their average body weight by 1 gram; the 12 birds given the prolactin in addition show an average increase of 21 grams; for the 3 birds given only prolactin this increase was 33 grams. The 12 birds of the 3 groups which received huge doses of F.S.H$^+$ appear to have gained weight; in fact they made average gains of from 20 to 31 grams in their reproductive organs (ovary + oviduct), and thus a loss of 20, 15 and 8 grams from their other somatic organs. The isosoluble fraction again caused no apparent increase in liver weight, but the 12 birds given prolactin in addition had livers 44 per cent heavier than the 17 birds injected with the isosoluble fraction alone.

SUMMARY

The pronounced anti-gonad action of prolactin in adult birds is an indirect effect of its action on the bird's own pituitary, where it stops the release of F.S.H.; thereafter the gonad undergoes the rapid and extensive atrophy which likewise follows complete hypophysectomy or sex hormone administration. The ability of estrone, progesterone and testosterone to suppress the F.S.H. output of the pituitary has been observed in adult male ring doves.

A measure of the normal daily output of F.S.H. from the pituitary of the adult male dove indicates this is equivalent to one minimum ovulation dose in the 4-month New Zealand rabbit.

Prolactin induced an increase in body weight in the several groups of adult male doves and female pigeons tested; in other similar groups such weight increase was partly or entirely overcome by preparations containing thyrotropic hormone. Thus 29 pigeons were dosed with an isosoluble pituitary fraction; the 12 birds of this group given also 15 to 60 units of prolactin daily for 6 days had livers 44 per cent larger than the 17 given the isosoluble fraction only. In 12 adult male doves liver size was increased 100 per cent or more by 50 units of prolactin daily for 10 days.

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THE LIBERATION OF ADRENIN AND SYMPATHIN INDUCED BY STIMULATION OF THE HYPOTHALAMUS

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Received for publication January 30, 1937

The investigations of Cannon and his co-workers have determined beyond question that during the widespread discharge of the sympathetic nervous system in emotional excitement, there is an increased secretion of adrenin which, liberated into the blood stream and distributed throughout the body, augments and prolongs the effects induced by direct nervous excitation.

More recently the existence of another sympatho-mimetic hormone "which diffuses into the blood stream when sympathetic nerves supplying autonomic effectors are stimulated,"[1] has been identified by these workers. The appearance of this substance, sympathin, has been demonstrated both during the direct (Cannon and Bacq, 1931; Rosenblueth and Cannon, 1932; Cannon and Rosenblueth, 1933, 1935) and the reflex (Liu and Rosenblueth, 1935) activation of sympathetic nerves, and has also been shown during the central activation of the sympathetic system in the hyperactive periods of the decorticate cat (Whitelaw and Snyder, 1934) and during emotional excitement in the intact animal (Newton, Zwemer and Cannon, 1931; Partington, 1936). A general review of this and related fields has been given by Bacq (1935).

The liberation of these two hormones, adrenin and sympathin, during emotional activity renders of interest the demonstration of their production on direct stimulation of the hypothalamic region of the brain, whose importance in sympathetic innervation and emotional excitement has been shown in many other ways (Karplus, 1928; Bard, 1929; Ranson and Magoun, 1933; Ranson, 1934a; Ranson, Kabat and Magoun, 1935; Kabat, Magoun and Ranson, 1935; Kabat, Anson, Magoun and Ranson, 1935; Ranson, 1936). The results of the present investigation, which has been directed to that end, confirm the demonstration by Houssay and Molinelli (1925) of the liberation of adrenin on stimulation of the hypothalamus and in addition reveal the liberation of circulating sympathin.

METHODS. The nictitating membrane (nm.) of the anesthetized cat (Dial, 0.6–0.7 mgm. per kilo) was used as an indicator for adrenin and sympathin in the manner devised by Rosenblueth and Cannon (1932).

For recording contractions induced by sympathin the smooth muscle of the nm. was denervated and sensitized by removal of the superior cervical sympathetic ganglion 15 to 20 days previously, some of the animals also being given cocaine, 4 to 7 mgm. per kilo. The adrenal glands in these animals were removed through a dorsolateral approach at the start or during the course of the experiment. For recording contractions induced by adrenin, the nm. was denervated at the start of the experiment, the animals being given only small doses of cocaine, 1 to 4 mgm. per kilo, or no cocaine at all.

The head of the animal was fixed, the upper and lower eyelids and the outer canthus were tied back, and the dorsolateral edge of the nm. was attached by a thread running over two pulleys to a writing lever giving an eight-fold magnification and exerting a tension of 4 grams on the membrane. Blood pressure was recorded in all experiments from the carotid artery on the side opposite the nm. studied. In the adrenalectomized animals it ranged between 80 and 140 mm. Hg; in the intact animals between 128 and 178 mm. Hg.

Stimulation of the hypothalamus was performed with the aid of the Horsley-Clarke instrument in the manner described by Ranson (1934b), the current being supplied by a single dry cell attached to a Harvard inductorium, the secondary coil of which was set at 8 cm. Some preliminary exploration was made in each animal until the best location was obtained; the electrodes were then usually left in place for the remainder of the experiment. Subsequent examination of the loci from which the effects were obtained showed them to be distributed in the different animals through the anteroposterior extent of the lateral hypothalamic area and the adjacent perifornical region. It is important to note that this is the area which other experiments have demonstrated as the main focus from which sympathetic responses may be obtained on direct stimulation.

Observations. *Sympathin.* A total of sixteen responses of the chronically denervated nm. obtained from four adrenalectomized animals provide data regarding the liberation of sympathin as a result of stimulation of the hypothalamus.

The characteristics of the response of the chronically denervated nm. to circulating sympathin are illustrated in figure 1. All of these responses were obtained in one experiment by stimulation for varying lengths of time of a single point in the hypothalamus with the same intensity of current and same frequency of induction shocks. A shows a contraction of 2 mm. lasting about 3 minutes, induced by a 6 second stimulus. B shows a contraction of 8.5 mm. lasting about 6 minutes, induced by a 15 second stimulus. C shows a contraction of 11 mm. lasting about 6½ minutes, induced by a 30 second stimulus. D shows a contraction of 14.5 mm. lasting about 10 minutes, induced by a 53 second stimulus. An

estimation of the latent period of these responses ranged between 20 and 35 seconds. The maximal contraction was reached in a little less than 2 minutes after the onset of the 6, 15, and 30 second stimuli; and in $2\frac{1}{2}$ minutes after the onset of the 53 second stimulus.

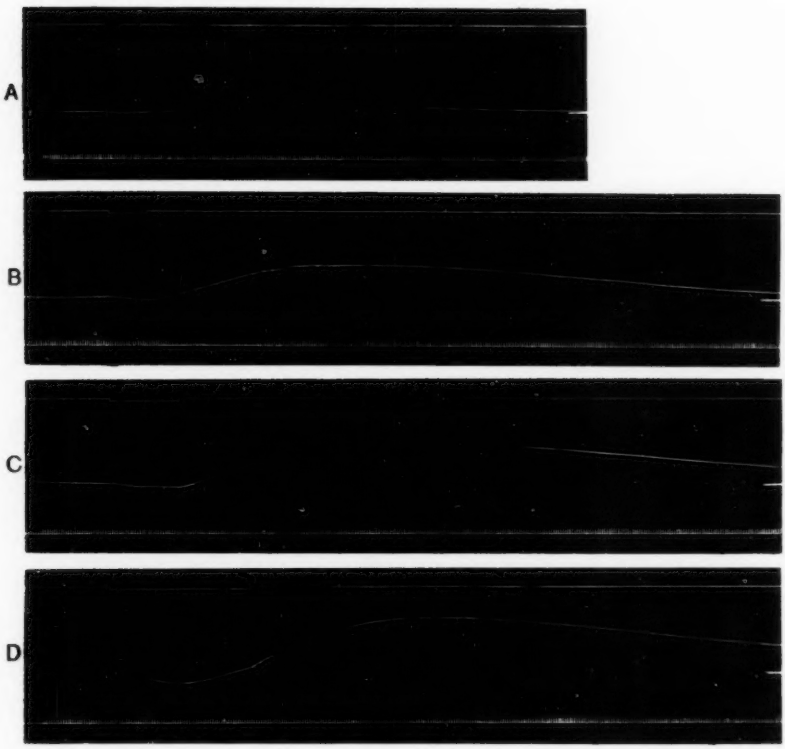

Fig. 1. Adrenals removed, no cocaine. The four records show the increasing contractions of the nictitating membrane, denervated 20 days, induced by sympathin liberated as a result of 6 (A), 15 (B), 30 (C), and 53 (D) seconds' stimulation of the same point in the lateral hypothalamic area at the level of the mammillary bodies. The dip in the upper line of each record represents the period of stimulation, the lower line shows the time in second intervals. The position of the base line has been indicated by a short white line at the right margin of each record. Only the first 5 minutes of tracings B, C, and D are shown.

Estimation of the latent period of contraction of the denervated membrane in such experiments was difficult because of slight shifts in tension appearing at the onset of hypothalamic stimulation. These consisted either of increases or decreases in tension and were probably artifacts due

to slight changes in position of the eyeball. In 2 responses in which they were altogether absent, periods of 13 and 22 seconds respectively intervened between the beginning of hypothalamic stimulation and the onset of contraction of the nm. An estimate of the latent period in 6 other responses showing very slight irregularities, ranged between 13 and 30 seconds. The average of these times is 18 seconds.

Once begun the contraction of the nm. was smooth and gradually progressed to a maximum, the height of which (magnified 8 times) ranged between 2 mm., induced by 6 seconds' stimulation of the hypothalamus, and 20 mm., induced by 2 minutes' stimulation of the hypothalamus, in each animal the height of contraction increasing directly with the duration of hypothalamic stimulation.

The higher the contraction the longer was the time usually required for its full development, and the time after the onset of hypothalamic stimulation at which the maximal contraction was reached became to some extent also a function of the duration of stimulus. With shorter stimuli, of 15 to 30 seconds' duration, this time ranged between 1 and 2 minutes; with longer stimuli, of 1 to 2 minutes' duration, it ranged between 2 and 3 minutes.

The peak of contraction rounded slowly and smoothly into a very gradual relaxation, the tracing approaching the baseline several minutes later and in such a slow fashion that an accurate determination of the end of contraction was difficult to make. Approximate measurements of the durations of contraction in these experiments showed them to vary between 3 and 10 minutes, in each animal the duration of contraction increasing as did its height with the length of hypothalamic stimulation.

Adrenin. The above observations indicate the impossibility of actually eliminating liberated sympathin in studying the release of adrenin during hypothalamic stimulation, and the problem becomes one of employing a differential indicator, insensitive to sympathin but responding to adrenin.

It was found that in animals in which the nm. was acutely denervated at the start of the experiment, and which were given only small doses of cocaine or no cocaine at all, pronounced contractions of the denervated nm. induced by stimulation of the hypothalamus could be obtained if the adrenals were intact, while after removal of the adrenals these contractions were either absent or so small as to be almost imperceptible, the nm. not being sufficiently sensitized to respond effectively to the liberated sympathin. It is concluded that the pronounced contractions are induced by adrenin liberated as a result of stimulation of the hypothalamus, and a study of 7 responses obtained from 3 animals with intact adrenals was made.

Estimation of the latent period of contraction to liberated adrenin was attended by the same difficulties as with sympathin, small shifts in the

baseline occurring at the onset of hypothalamic stimulation. Three responses in which the baseline was steady showed a period of 16, 24 and 30 seconds respectively intervening between the onset of stimulation and the beginning of contraction of the denervated nm. Estimates of the latent period in the other responses varied between 16 and 25 seconds. The average of these times is 22 seconds.

Once begun, the contraction of the nm. induced by adrenin progressed slowly to a maximum, the peak height (magnified 8 times) ranging between 5 and 15 mm., and being reached between 50 and 90 seconds after the onset of stimulation. The higher contractions, induced by the longer

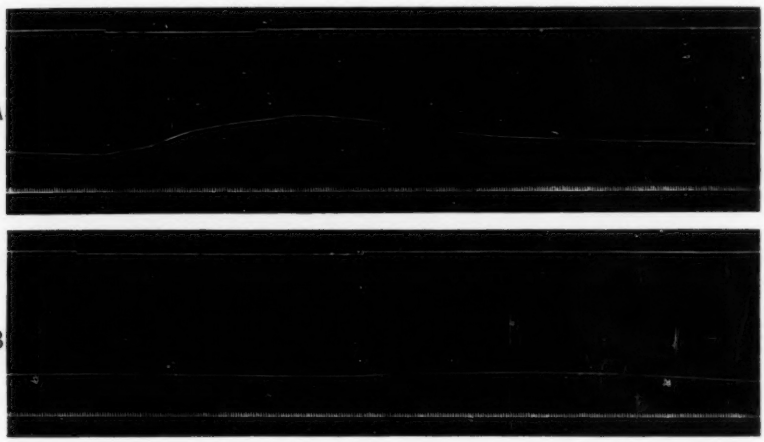

Fig. 2. Nictitating membrane denervated at the start of the experiment. 4 mgm. cocaine per kilo. Record A, with the adrenals intact, shows a contraction of the nm. induced by adrenin, liberated as a result of 61 seconds' stimulation of a point in the lateral hypothalamic area at the level of the mammillary bodies. Record B shows the absence of response to 117 seconds stimulation of the same point in the hypothalamus after removal of the adrenals.

periods of hypothalamic stimulation, required the longer periods of time to reach their maximum. The relaxation of the nm. was gradual, the approximate duration of contraction ranging between $1\frac{1}{2}$ and 4 minutes.

The characteristics of the response of the acutely denervated nm. to liberated adrenin are illustrated in figure 2A, which shows a contraction of 9.5 mm. lasting about 4 minutes, induced by 61 seconds' stimulation of the hypothalamus. Control stimulation of the same point in the hypothalamus for 117 seconds, about twice the previous duration, after removal of the adrenals is shown in figure 2B, the nm. showing an almost negligible contraction of 0.5 mm.

The blood pressure before stimulation in figure 2A was 138 mm. Hg and hypothalamic stimulation induced an increase of 92 mm. Hg. After removal of the adrenals three stimuli with an initial blood pressure of 108, 95, and 88 mm. Hg gave increases in pressure of 100 (1 min. stimulus), 125 (1 min. stimulus), and 160 (2 min. stimulus) mm. Hg, respectively, in each instance the nm. remaining essentially quiescent. It is clear that the operative procedure involved in removing the adrenals depressed the resting blood pressure level somewhat, but by no means to a level of shock. The striking pressor reactions obtained after adrenalectomy also oppose a conception of the animal's being in a depressed condition.

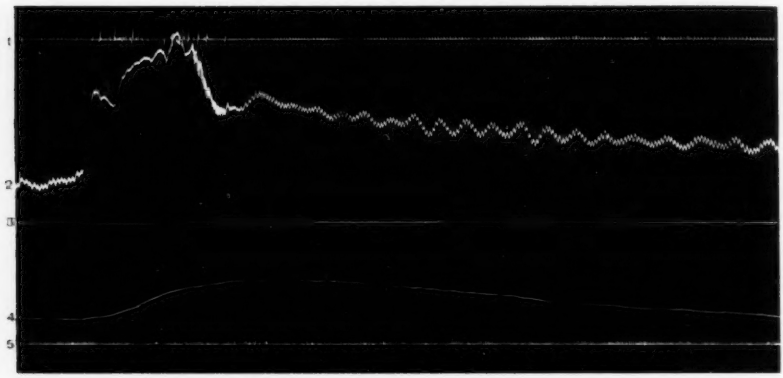

Fig. 3. Adrenals removed, 4 mgm. cocaine per kilo. The tracings from above downward represent *1*, respiration from tracheal cannula; *2*, arterial pressure from left carotid; *3*, signal marker with the dip indicating the 61 second period of stimulation of a point in the lateral hypothalamic area at the level of the caudal edge of the optic chiasma; *4*, right nictitating membrane, denervated 19 days, and *5*, the time in second intervals. During hypothalamic stimulation the blood pressure rose from 108 to 200 mm. Hg. Compare the prolonged hypertension following the cessation of hypothalamic stimulation with the contraction of the denervated nm. induced by liberated sympathin.

Hypothalamic stimulation in other experiments under conditions comparable except for senitization of the nm., have yielded clear-cut contractions of this structure (figs. 1 and 3). The only way, therefore, in which we can account for the absence of contraction of the nm. in figure 2B (adrenals removed) is to consider its acutely denervated smooth muscle insufficiently sensitized to respond to the liberated sympathin, and from this point of view we would ascribe the contraction shown in figure 2A (adrenals intact) to adrenin liberated as a result of hypothalamic stimulation.

Relation to other effects of hypothalamic stimulation. The long latency

and delay in maximum contraction of the nm. produced by adrenin and sympathin in these experiments definitely indicate that the circulation of these hormones is not responsible for the initial effects of hypothalamic stimulation, though their accumulation as stimulation is continued must certainly increase the magnitude of the other responses obtained. This effect would appear to merge indistinguishably with that induced by direct nervous connections, and it is not possible, for example, to point out a part of the pressor reaction during hypothalamic stimulation as that for which adrenin is solely or chiefly responsible.

A large number of the hypothalamic pressor responses are characterized, however, by a persistent and slowly declining hypertension following the cessation of stimulus, and the possibility that this is maintained in large part at least by a humoral agent may be briefly considered. While adrenin may contribute to the maintenance of this after-elevation of blood pressure, the effect is readily obtained in the absence of the adrenals, and the question arises as to whether it can be attributed to sympathin. The effect can be obtained without sensitization of the animal; and while smooth muscle, which has been sensitized, is conventionally utilized as an indicator for sympathin, Cannon and Uridil (1921) have demonstrated a hepatic pressor substance, which Cannon and Rosenblueth (1933) subsequently identified as sympathin, in animals not subjected to any sensitizing process. Cannon and Bacq (1931) have demonstrated also the pressor effect of sympathin released on stimulation of the sympathetic nerves to the tail region in non-sensitized animals. Many of the records obtained in the present experiments, as that illustrated in figure 3, show a rough parallel between the persistent hypertension following the cessation of hypothalamic stimulation and the prolonged contraction of the denervated nm. produced by liberated sympathin, but this parallelism is so rough as to appear indicative rather than conclusive.

If this prolonged elevation of arterial tension could be obtained only after stimulation of a central sympathetic center in the hypothalamus, the possibility that it might be the expression of a central after-discharge should be seriously considered. Johnson and Johnson (1931), however, have observed what is apparently the identical phenomenon following stimulation of the peripheral end of a sectioned splanchnic nerve under conditions which eliminate after-discharge, reflex effects, cardiac stimulation, splenic contraction, or the liberation of adrenin from the adrenal glands as responsible factors. They consider three agencies, adrenin from chromaffine tissue, peripheral vascular rhythm, and the liberation of sympathin, as possibly responsible for the phenomenon, but to us the weight of their evidence would appear to rest upon sympathin.

The chief interest, however, in the demonstration by Houssay and Molinelli (1925) of the liberation of adrenin, and in the demonstration in

the present experiments of the liberation of adrenin and sympathin in response to hypothalamic stimulation lies in the light thrown upon the compound mechanism by which a central representation of the sympathetic system in the brain stem exerts its influences upon the body. That the hypothalamus produces changes throughout the body by way of direct nervous connections is well known. The demonstrations of these hormones released on hypothalamic stimulation make it evident also that the effects elicited by direct nervous influences are supplemented by and summed with those induced by circulating humoral substances, which act in the same way as the nervous influences that cause their release.

SUMMARY

Using the denervated nictitating membrane of the anesthetized cat as an indicator, the liberation of circulating adrenin and sympathin in response to stimulation of the hypothalamus has been demonstrated.

The prolonged latent period and delay in maximal contraction of the denervated membrane induced by these substances, indicates that they are not responsible for the initial effects of hypothalamic stimulation upon other organs. Their rôle in augmenting and prolonging hypothalamic effects induced by direct nervous connections is discussed and it is concluded that the rôle of the hypothalamus in visceral innervation and emotional excitement is effected both by direct nervous influences and by humoral substances for whose release these nervous influences have been responsible.

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THE INHIBITION OF ESTROUS CYCLES IN THE ALBINO RAT BY PROGESTERONE

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Received for publication February 1, 1937

Since Beard (1897) and Prenant (1898) suggested that the corpus luteum is responsible for the inhibition of ovulation during pregnancy, numerous attempts have been made to establish this function and to isolate the active principle. Loeb (1911) and subsequent workers demonstrated the inhibiting function of the corpus luteum by experiments involving the removal of this tissue from the ovaries of pregnant and non-pregnant animals. Pearl and Surface (1914) and other workers reported the inhibition of estrus or ovulation using aqueous extracts of corpora lutea, while Loeb (1917) and Corner and Hurni (1918) using aqueous extracts failed to inhibit estrus. Papanicolaou (1926) and a number of others inhibited ovulation with fat-soluble extracts of lutein tissue. Parkes and Bellerby (1927) and Winter (1930) found that a thermolabile active principle could be obtained from an acetone-soluble fraction of an ether extract of corpora lutea. Payne, Peenan, and Cartland (1928) reported inhibition using an unsaponifiable fraction of corpus luteum tissue. Meyer (1932) found that acid alcohol extracts of corpora lutea, of testicular and of muscular tissue, as well as certain dyes would inhibit estrous cycles. Zondek (1927) and Del Castillo (1928) reported inhibition by feeding thallium. Recently, Robson (1936) has reported inhibition using testosterone.

Although in many of these experiments extracts of corpora lutea have been reported to delay the estrous cycle, it will be noted that positive results have been obtained not only with many different fractions but also with extracts of other organs and with organic and inorganic chemical substances. It is obvious that experiments purporting to show a specific effect of the corpus luteum must be conducted in such a way as to rule out nonspecific and merely toxic effects. The hormone injected must be chemically pure, and toxic action must be ruled out by prompt return of normal rhythm after the period of experimental treatment.

The isolation of the corpus luteum hormone in pure form by Allen and Wintersteiner (1934) has made such experiments possible. Lahr and Riddle (1936) injected six rats with 0.03 mgm. of crystalline progesterone daily

for 12 to 16 days and observed no interruption of the estrous cycle; using 0.3 mgm. daily they noted slightly prolonged estrus in several animals. Selye, Browne and Collip (1936) report suppression of estrus using 4 mgm. of progesterone daily. Makepeace, Weinstein, and Friedman (1936) have inhibited ovulation in the rabbit, using crude progestin-containing extracts and pure progesterone in amounts equal to or greater than that required to maintain pregnancy in a castrate rabbit.

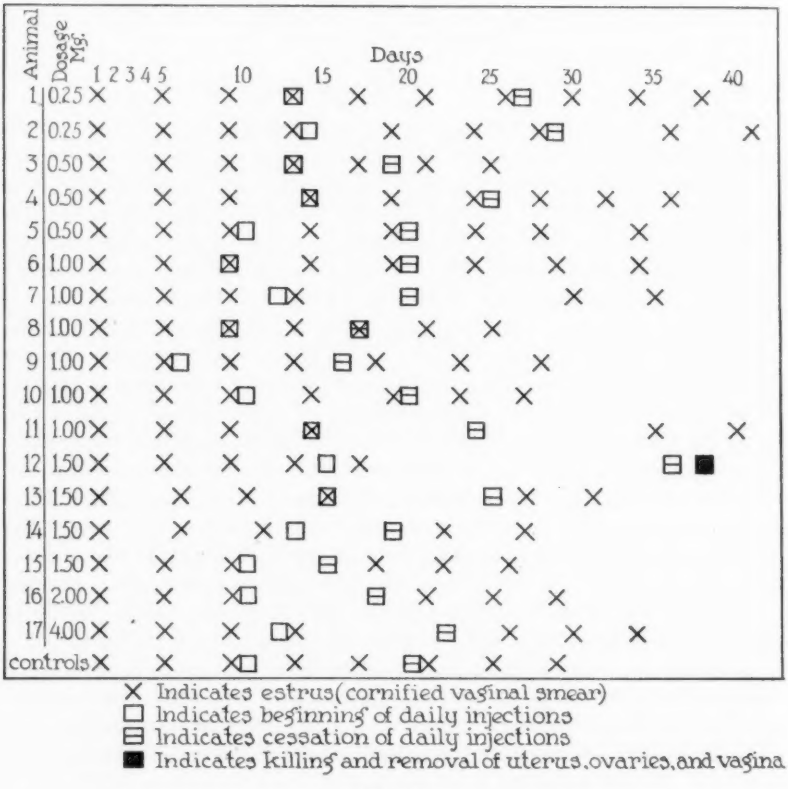

Fig. 1

For the present series of experiments mature female albino rats weighing 150 to 200 grams each were selected. Prior to and during the experiments these animals were fed calf meal from self-feeders and were given lettuce at regular intervals. The estrous cycle in each rat was followed using the vaginal smear method of Long and Evans (1922). Samples were taken from the vagina with a spatula at about the same time every day, stained with methylene blue, and studied microscopically. After regular normal

estrous cycles were established, the animals received daily subcutaneous injections of 0.1 cc. of sesame oil containing crystalline progesterone dissolved in a few drops of benzene. Six controls were run using benzene in sesame oil. Animals received injections of crystalline progesterone ranging from 0.25 to 4.0 mgm. ($\frac{1}{4}$ to 4 international units) daily. The progesterone used in animals 1, 2, 3, 4, 5, 9, 10, 11, 12, 13, 14, 15, 16 was alpha progesterone as prepared by Allen and Goetsch (1936); animals 6, 7, 8, 17 received synthetic alpha progesterone as prepared by E. Merck and Company.

The results are shown in the figure. In the majority of the cases 0.25, 0.5, and 1.0 mgm. did not inhibit the estrous cycle but prolonged it slightly. In two animals (nos. 7 and 11), receiving 1.0 mgm. the estrous cycles were inhibited, but did not return to normal rhythm within a reasonable time following cessation of injection. The initial inhibition may have been due to progesterone, but it is probable that the continued inhibition was due to some other factor. In the animals receiving 1.5, 2.0, and 4.0 mgm. of progesterone daily estrous cycles were uniformly interrupted, but were resumed 3 to 5 days following the last injection. It was found that injections made 3 days before the expected estrus inhibited that estrus if the dosage of progesterone were adequate; but injections made less than 3 days before the expected estrus, although the dosage was adequate, failed to inhibit that estrus. This may indicate the rate of absorption of crystalline progesterone in sesame oil from subcutaneous tissues.

Rat 12, inhibited for 21 days, was killed when the vaginal smear indicated proestrus (following the cessation of injections). The ovaries showed large corpora lutea and a few large Graafian follicles. Both horns of the uterus were about 1 cm. in diameter as compared with the average normal diameter of 3 mm. Microscopic examination revealed a decidуoma. The vagina disclosed typical proestrous structure.

The relatively large dosage required to produce inhibition indicates the improbability that progesterone (or at least progesterone alone) is responsible for the normal inhibition of estrus and ovulation during pregnancy in the rat.

SUMMARY

Crystalline progesterone in doses 1.5 mgm. and greater inhibited estrous cycles in normal female albino rats; smaller doses failed to do so.

I wish to express my appreciation for the many valuable criticisms and suggestions that Dr. George W. Corner and Dr. Willard M. Allen have so kindly given.

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A CHEMICAL STUDY OF THE BLOOD OF THE DEVELOPING CHICK[1]

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Received for publication February 3, 1937

Within the last few years increasing attention has been given to studies concerning the changes in the concentration of a number of blood constituents during prenatal development. Such studies have become possible as a result of the development of chemical methods which require only a fraction of a cubic centimeter of blood for analysis. Data of this nature are of interest since by observing the changes in the blood occurring during the embryonic period, further information may be obtained relating to the metabolism of the various foodstuffs. In addition, since it is possible to determine histologically the extent of functional development of the various organs of the embryo at a given age, such chemical blood studies may also throw light upon the influence of a given organ on intermediary metabolism. With these points in mind, the present investigation was undertaken.

METHODS. Eggs from white leghorn hens were employed throughout this study, and the incubation process was carried out under the optimum conditions (1). After hatching, the chicks were kept in the warm incubator with free access to water but no food until they were three days old. After that time they were fed a suitable commercial grain mixture and were gradually accustomed to ordinary room temperatures. Therefore, except in the case of the determinations that were made on those chicks which were sacrificed 29 days after the beginning of incubation, the figures which are here reported represent the analyses of the blood of starved animals.

Eggs incubated for the desired period were carefully opened and sufficient blood for analysis was withdrawn from the vitelline artery or vein into a 1 cc. tuberculin syringe. The blood from the hatched chicks was

[1] A report of the work given in this paper was presented before the Cleveland Section of the Society for Experimental Biology and Medicine on November 13, 1936 (Proc. Soc. Exper. Biol. and Med. **35**: 451, 1936).

The data presented in this paper are taken from the dissertation submitted by Carla M. Zorn to the Graduate School of Western Reserve University, June 1936, in partial fulfillment of the requirements for the degree of Master of Science.

removed from the jugular vein after the animals had been anesthetized with ether. From control experiments it was found that the very short period of anesthesia employed caused no appreciable changes in the concentration of the blood constituents studied. Since it was found that satisfactory blood samples could not be obtained from embryos of less than nine days' incubation no analyses were attempted until that time. From this period on, however, the analyses were carried out throughout the entire incubation period and for several days after hatching.

The following blood constituents were studied: sugar, uric acid, cholesterol, hemoglobin, and red blood cells. The blood sugar concentration was determined by the Jeghers and Myers' (2) modification of Folin's micro method (3). The concentration of uric acid was determined according to the micro method of Folin (4). A micro modification of the Myers and Wardell (5) method was employed to determine the concentration of cholesterol. Hemoglobin concentrations were determined by the acid hematin method employing the Newcomer disc as a standard. The red blood cell counts were made in a standard hemacytometer with a Levy counting chamber containing the Neubauer rulings, using a corresponding dilution pipette.

Results. Data of the type obtained in this study are conveniently presented with the aid of graphs. The changes occurring in the blood sugar, uric acid and cholesterol concentrations are presented in figures 1, 2 and 3, while the changes in the hemoglobin concentration and erythrocyte counts are shown in figure 4. The points on these charts represent average values in every case and the vertical lines indicate the range which was encountered in the individual determinations. The number of determinations from different embryos used to compile the average value is indicated above the respective vertical line. The horizontal dotted line represents the average concentration of the constituent in a full grown chicken as determined by Dyer and Roe (6).

Blood sugar. From an examination of figure 1 it will be seen that the range of variation in the concentration of blood sugar on any one day of incubation is quite wide. However, from the 9th to the 15th day of incubation the average amount of reducing substances present in the blood is seen to be quite small compared to that present in the adult chicken. During this period few significant changes are to be observed in the average values. It was found that from the 16th day of incubation to hatching, the average values are equal to or above those found in the adult chicken. Further, shortly after hatching the concentration of blood sugar still remains far in excess of the normal adult level of 157 mgm. per 100 cc.

Uric acid. It will be seen from the results presented in figure 2 that the concentration of blood uric acid gradually increases from the 9th day

of incubation until hatching. The increase in uric acid, therefore, is demonstrated earlier than that of blood sugar. At the end of the incubation period the adult average level of 4.2 mgm. per 100 cc. is reached. It

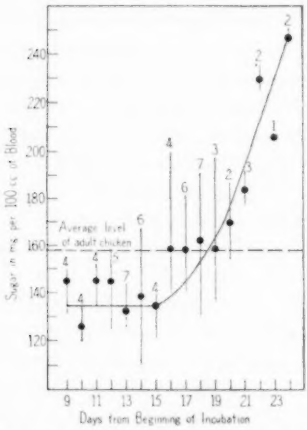

Fig. 1. The changes in the blood sugar concentration during development.

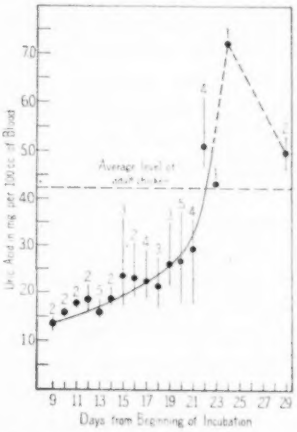

Fig. 2. Uric acid concentration of the blood during development.

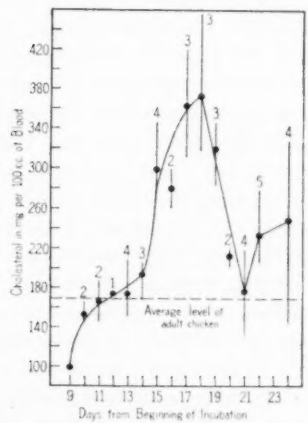

Fig. 3. The changes in the blood cholesterol concentration during development.

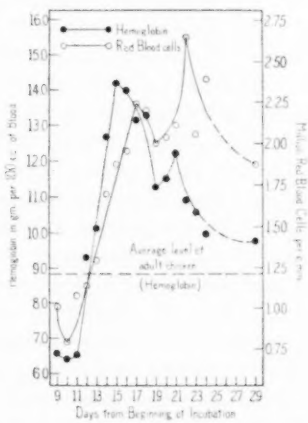
Hemoglobin
Red Blood cells
Hemoglobin in gm. per 100 cc. of Blood
Average level of adult chicken (Hemoglobin)
Days from Beginning of Incubation

Fig. 4. Hemoglobin and red cell concentration of the blood during development.

will be seen that a high level of 7.2 mgm. was obtained on the 24th day of development. This represents the result of a single determination. Inasmuch as Dyer and Roe have reported uric acid concentrations of this

order as upper normal values in the blood of hens, a broken line has been drawn to construct the part of the curve passing through this point to indicate its questionable significance.

Cholesterol. As illustrated in figure 3, the blood cholesterol is related to the developmental period in a manner different from that observed with blood sugar and uric acid. Thus, with the exception of a plateau between the 10th and the 13th days, the curve rises steeply to a peak at the 18th day which represents an average value of 364 mgm. per 100 cc. of blood. The curve then falls sharply. On the 21st day, coincident with the termination of the incubation period, an average cholesterol value of 170 mgm. is observed, which approximates the normal adult level. Immediately after hatching the blood cholesterol again increases, reaching a level of 240 mgm. per 100 cc. of blood. This cholesterol level persisted throughout the period of observation.

Hemoglobin and red blood counts. In view of the possible interrelationship between the hemoglobin and the erythrocyte concentrations, the results of the analyses of these two constituents have been considered together and are presented in figure 4. The curves have several characteristics in common. Until the 11th day of incubation very low values are found in the case of both hemoglobin and erythrocyte contents. At this time, however, the concentrations of both constituents are found to increase very markedly. Thus the hemoglobin reaches a high average value of 14.1 grams per 100 cc. on the 15th day. The number of red cells continue to increase until the 17th day, when the value of 2.2 million per cu. mm. is attained. The initial increase of both constituents is followed by a definite fall in the level during the hatching period. A temporary but definite recovery is again noted within a day after hatching. However, in the case of hemoglobin the recovery is only partial, the highest average value after hatching being only 12.2 grams with the values on subsequent days indicating a gradual decrease in the concentration towards the normal level for the adult fowl. The recovery in the red blood cell count is more marked and reaches a level of 2.61 million per cu. mm. of blood the day after hatching which is higher than that encountered at any other time during the period of ontogenesis under consideration. However, as in the case of hemoglobin, this level is not maintained and seven days later the count may be noted to have decreased to 1.8 million.

Discussion. The use of the chick embryo in the study of the blood chemistry during development has certain advantages which have been mentioned above. However, fertilized ova of the fowl, retained in the oviduct for varying lengths of time, may be subjected to rather wide differences in external temperatures after laying. These factors, beyond experimental control, induce a small and unpredictable variation in the age of embryos incubated for the same period. The data presented in

the charts show that the progressive changes in concentration of the constituents of the blood may take place very rapidly during certain periods of development. Small variations from the specified age caused by the factors mentioned above would tend to alter slightly the results obtained during such periods. It seems logical, therefore, to consider only those curves constructed from average figures of daily changes as of real significance.

The use of the normal adult values of Dyer and Roe (6) for the Plymouth Rock breed as a basis of comparison with our results from White Leghorns may appear to be ill advised. However, the range of normal variation found by Dyer and Roe for blood sugar, hemoglobin, uric acid and cholesterol appears great enough to overshadow any variations due to the use of different breeds.

In studies of the blood sugar of the developing chick Hanan (7) and Vladimiroff and Schmidt (8) both found a constant level in the concentration of this constituent from the 11th to the 19th or 20th day of incubation, although the respective levels reported by these authors are not in accord. Vladimiroff later (9) repeated this work and recapitulated the findings of Vladimiroff and Schmidt (8). The data of the present study show that the general level of blood sugar concentration agrees with Vladimiroff and Schmidt rather than with Hanan, but on closer examination the data do not entirely bear out the conclusions arrived at by the former authors. Thus, although the concentration of blood sugar remains fairly constant during the early part of the incubation period studied, average values equal to those of the adult are found as early as the 16th day. On the 20th day and continuing after hatching a rapid rise occurs which, 3 days after hatching, reaches a value far above that given for the adult.

Other workers, Riddle and Honeywell (10) with pigeons, and Britton (11) with kittens, have also found evidence of a rapid rise in blood sugar concentration after birth. The results of the present study therefore are in accord with the results of similar investigations, with the exception that in the present case, a gradual rise to the adult level during development is indicated, rather than a sudden elevation to adult values.

Changes in the concentration of uric acid in the blood during the development of the chick have not, to our knowledge, been reported in the literature. Therefore, no comparison of the results of this study with other values is possible. The results indicate that a gradual rise in uric acid concentration occurs from the 9th day of incubation until hatching. After this time the uric acid concentration rises more markedly and soon reaches the adult value.

It is of interest to compare the present results on the uric acid concentration of the blood with those of Fiske and Boyden (12) and Needham

(13) on the milligrams of uric acid excreted per day per embryo. The results of these workers are in accord with those reported here in that their values indicate an increasingly rapid formation of uric acid in the embryo which is well under way by the 9th day of incubation. Since it is fairly certain that the liver is the most important site of uric acid formation in the fowl, it seems reasonable to suggest that the chick embryo liver has acquired the capacity to form uric acid previous to the 9th day of incubation.

Vladimiroff and Schmidt (8) have carried out determinations of the total lipid content of the blood of chick embryos from the 11th day of incubation until two days after hatching. These authors noted that the concentration of lipid material in the blood at first increases with the age of the embryo, reaching its highest value on the 17th and 18th days. Our values for blood cholesterol are in agreement with their observation. It should be pointed out, however, that the changes in the cholesterol concentration reported here do not follow a course which is in every respect parallel with the curve for total fat. Thus, Vladimiroff and Schmidt observed an increase of about 150 mgm. per 100 cc. in the total lipid content from the 11th to the 17th day of incubation, while we have found a more marked rise of 200 mgm. per 100 cc. for cholesterol during the same period. Further, the temporary fall in the cholesterol curve at the time of hatching is not reflected in the curve for total lipids reported by the above workers. In contrast to the partial recovery in the cholesterol content of the blood of hatched chicks encountered in the present study, Vladimiroff and Schmidt demonstrated a gradual but continuous downward trend in the blood lipid concentration during the last days of incubation which continued for the first few days after hatching.

It is difficult to consider the results relating to the changes in hemoglobin concentration in the light of other published values. This is due to the fact that a number of different methods of expressing hemoglobin concentration have been employed by various investigators. Liebermann (14) expressed his results as per cent of total body weight. Sendju (15) determined the milligrams of hemoglobin in the total blood volume of the embryo. The results of Sümegi (16) are expressed as per cent of the normal. The values determined in the present study have been recorded in the more desirable terms of grams per 100 cc. of blood.

In spite of this difficulty, certain correlations may be pointed out. Thus, the results of the limited number of determinations of Liebermann indicate that although the actual amount of hemoglobin in the embryo increases with age, its concentration when expressed as per cent of total body weight is lowered at the time of hatching. This is similar to the changes observed in figure 4. The most recent investigator along this line, Sümegi has expressed his results as per cent of the normal value.

The curve in this case is not complete, the first analyses having been carried out on the 15th day of incubation, but the temporary decrease in the hemoglobin concentration noted at the time of hatching is again evident.

A comparison of the hemoglobin concentration of the developing organism with that of the adult demonstrates the relatively high hemoglobin concentration during the last third of the incubation period, an observation in keeping with the results repeatedly observed for many species of animals.

The available data in the literature relating to the erythrocyte concentration in the blood of the developing chick are limited to the early work of Ascarelli (17) and the much more recent investigation of Sümegi. The results of these two workers are roughly comparable with ours, both demonstrating a lowered erythrocyte concentration near the end of the incubation period followed by a secondary increase.

A comparison of our curve for hemoglobin and erythrocyte concentrations with the work of Dawson (18) on the erythrocytes of the developing chick may be of interest. It is well known that young, immature erythrocytes contain a relatively small amount of hemoglobin compared with the more mature cells. Further, when new centers of erythropoiesis become active, numerous relatively immature erythrocytes enter into the circulation. Dawson finds a transitory rise in the number of immature erythrocytes in the circulation during the period from the 12th to the 14th days which he attributes to the early functioning of the spleen and bone marrow as hematopoietic centers. One would expect the curve for erythrocyte concentration to rise more rapidly than that for hemoglobin concentration during this period. However, we have not found this to be the case; in fact, it is during this period that the curve for hemoglobin concentration is relatively higher than that for erythrocyte concentration.

Except where the inferences have been fairly obvious, few conclusions have been drawn from the results of this investigation. Further work is in progress to determine the significance of the changes in the concentration of the various blood constituents studied.

SUMMARY AND CONCLUSIONS

The progressive changes in concentration of blood constituents of the chick embryo have been investigated from the 9th day of incubation until the third day after hatching.

The following changes appear to have been fairly well established:

1. The concentration of blood sugar is maintained at a reasonably constant level during the early part of the period studied. A rise toward the adult value, first noticeable on the 16th day, becomes very marked at

the time of hatching and reaches a level which is definitely above the average normal adult concentration.

2. The values for uric acid content of the blood rise continually during the period of incubation investigated. Shortly after hatching, the values reach levels comparable to those of the adult.

3. The concentration of blood cholesterol increases rapidly from the 9th to the 18th day of incubation. A gradual decrease in the values follows this rise and they reach the normal adult level at the time of hatching. A secondary increase occurs immediately thereafter. Thus, a hypercholesterolemia with respect to the normal adult value is present during the greater part of the period which has been investigated.

4. The changes in the hemoglobin and erythrocyte values are roughly parallel. The concentration of both constituents increases directly with the age of the embryo, with the exception of a temporary decrease at hatching. The values for hemoglobin in the embryo are elevated above those of the normal adult during the latter third of the developmental period.

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THE RELATION OF CYCLIC CHANGES IN HUMAN VAGINAL SMEARS TO BODY TEMPERATURES AND BASAL METABOLIC RATES[1]

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Received for publication February 4, 1937

A detailed study of the human ovulation cycle is increasingly valuable the closer we come to establishing reliable criteria. Hartman's recent monograph (5) gives an excellent survey of the relevant literature. For the investigation of actual ovulation time direct methods, namely, recovery of ova or the finding of recent corpora lutea are possible only on laparotomy; bimanual palpation of the ovaries (6) cannot be effective in human subjects. Indirect methods are morphological (biopsies or smears), chemical (isolation and assay of hormones), and physiological (demonstration of cyclic variants of metabolism dependent upon rhythmic changes in the gonads). Of the indirect methods only those techniques can be employed which are acceptable to the non-scientific mind of the subjects. Biopsies or repeated withdrawals of blood like hormone excretion studies are excluded. Similar reasons exclude detailed studies of water and mineral metabolism and of uterine motility. The present report is limited, therefore, to data obtained from vaginal smears, body temperatures, basal metabolic rates, blood pressure, and pulse rates.

Papanicolaou's study of human vaginal smears (8) prepared after the guinea-pig vaginal smear method of Stockard and Papanicolaou (9) showed that, unlike the guinea-pig smear which consists at oestrus only of cornified epithelial cells, the comparable human smear shows relatively little desquamation of cornified and non-cornified epithelial cells, no mucus, and freedom from leukocytes and bacteria (fig. 1d). The name "ovulative" for this phase is justified by the fact that it is associated with a freshly ruptured Graafian follicle in all women on whom the performance of laparotomy permitted exploration of the uterine adnexae. Further evidence that ovulation occurs during this smear phase is offered by recent publications on sterility by Meaker (7). Comparing observations in guinea pigs and human smears from other parts of the ovulation cycle, it

[1] Preliminary report in Proc. Soc. Exper. Biol. Med. **35**: 618, 1937.

[2] Fellow of the General Education Board.

is recognized that postovulative extreme leukocytic invasion of the vagina is present in both (fig. 1e). In women the cornified cells disappear gradually during the entire postovulative-premenstrual period which is termed secretory to keep the terminology comparable with that customarily applied to the uterus of this stage (vide Evans and Swezy, 4). The premenstrual phase is characterized by abundant mucus, leukocytes, bacteria and epithelial cells with dense nuclei and relatively little poorly staining

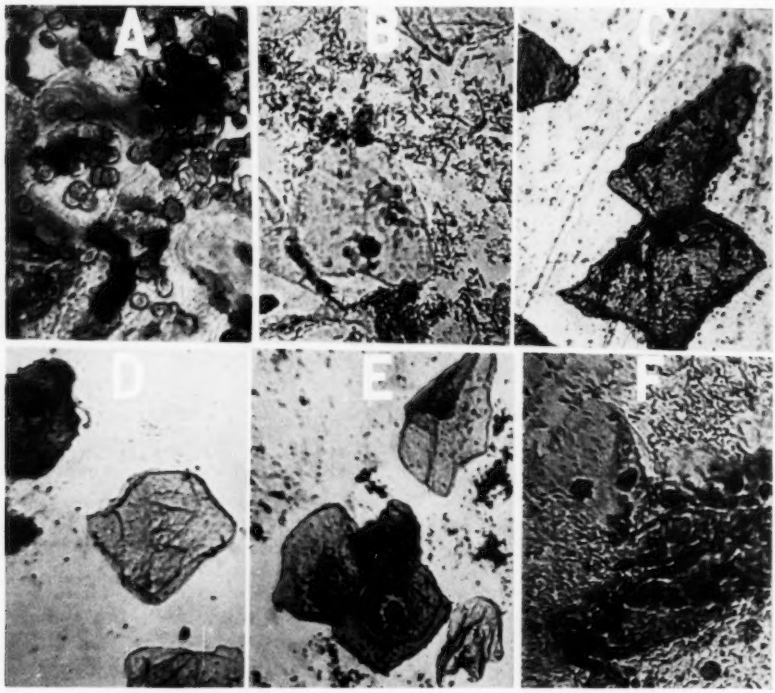

Fig. 1a. *Menstrual smear* showing chiefly masses of erythrocytes obscuring epithelial cells, leucocytes and strands of mucus. Day 3 (27-day cycle).

b. *Postmenstrual smear* showing extreme bacterial invasion and numerous leukocytes. Day 8 (31-day cycle).

c. *Preovulative smear* showing both cornified and uncornified epithelial cells, few leukocytes or bacteria, but abundant mucus. Day 13 (28-day cycle).

d. *Ovulative smear* showing but few cells; these are cornified or cornifying epithelial cells. Day 14 (28-day cycle).

e. *Postovulative smear* showing return of bacteria and leukocytes and the persistence of cornified cells. Day 18 (27-day cycle).

f. *Premenstrual smear* showing a clump of cornified cells, the reappearance of the normal squamous cells, and the abundance of bacteria and leukocytes. Day 23 (28-day cycle).

cytoplasm (fig. 1f). The menstrual smear (fig. 1a), apart from its blood, is very like the premenstrual smear but that the epithelial cells are smaller. In the postmenstrual phase (fig. 1b) there is an invasion of the vagina by hosts of bacteria followed within twenty-four hours by an outpouring of leukocytes. The preovulative or "copulative" phase which follows shows relatively few bacteria and leukocytes (fig. 1c) but mucus and epithelial cells with vesicular nuclei and granular cytoplasm in abundance and few cornified cells. This completes the cycle.

If an untreated erosion of the cervix is present, the smears are modified by the presence throughout the cycle of more polymorphonuclear neutrophilic leukocytes than in otherwise normal subjects. Nevertheless, while the various phases of the cycle blend into each other, and while it takes considerable experience to distinguish them, the typical smears can be clearly defined, and constitute to date the best means, short of operative procedure, of determining ovarian activity. Vaginal smears are made each morning by the subject, fixed in 50 per cent ether–50 per cent alcohol at once, and brought to the laboratory where they are stained with hematoxylin and eosin. This report summarizes approximately 1200 smears.

Body temperatures are known to vary during the menstrual cycle. Van de Velde (10) suggested a relation between gonad function and temperature but failed to demonstrate it. Subsequent work (12) has confirmed the existence of such a cycle and has made its exact relationship with gonad function a problem of importance. Body temperatures, taken with clinical thermometers, are registered by the subjects before rising from bed in the morning, i.e., usually about 6 a.m. Temperatures taken thus are comparable for Kleitman and co-workers (11) have shown that the body temperature in healthy males varies but very slightly from day to day, if it be taken at approximately the same time, and under constant conditions.

Basal metabolic rates are determined in duplicate eight minute periods with the usual precautions concerning the basal condition. The Benedict-Roth apparatus is used and the soda-lime changed often enough to make sure that no error is introduced. No data are acceptable unless the duplicates check within 5 per cent; the lower reading is considered the more nearly accurate.

Blood pressure is determined by the auscultatory method with three to five readings taken at one-minute intervals between the duplicate basal metabolic periods. Average values for systolic and for diastolic pressure are used.

Pulse is counted for two alternate half-minute periods and the average value recorded.

Each subject keeps a diary in which diet, activities, emotional states,

pain and other subjective sensations are recorded. Only two subjects record any mid-period pain (Mittelschmerz) but in these it is regular and coincides in time with the ovulative smear.

As the basis of this report sixteen normal women (ages twenty-one to thirty-nine years) without pelvic abnormality have been selected as subjects. Their coöperation is assured by their interest in the problem as medical or graduate nursing students. The period of study for the first four subjects has been sixty-one consecutive days during the fall of 1935, then one hundred and twenty-four consecutive days in the winter and spring of 1936, and finally thirty consecutive days last summer. The entire group with a single exception has been studied for about sixty days in the autumn of 1936.

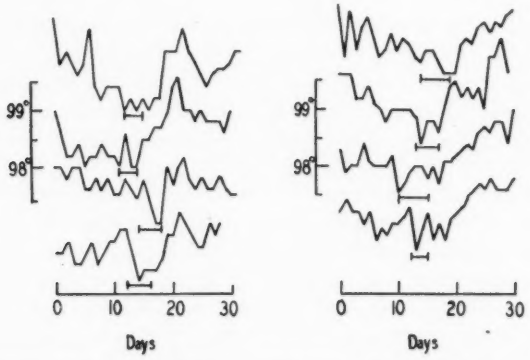

Fig. 2. Typical set of temperature curves of two subjects. Each curve starts with the first day of menstrual flow, and ends on the first day of the next cycle. The bar beneath each curve represents the duration of the preovulative phase.

Figure 2 shows two sets of typical temperature curves. Each curve begins with the first day of menstruation and ends with the first day of the following month. It can be seen readily that all the curves have a trough near their midpoint and a peak just before the recurrence of menstruation. The bar below each curve represents the duration of the preovulative phase of the menstrual smear, terminating with the ovulative smear. The correspondence between the trough of the curve and the preovulative bar is clear.

Table 1 summarizes the temperature data, and shows that the temperatures at ovulation are significantly lower than those of the premenstrual period and even than those of the rest of the cycle. In some cycles, the range of temperature variation is greater than in others. The range of variation is usually more than 1.5° Fahrenheit. The variability prevents recognition, in advance, of the lowest point in the trough of the

temperature curve though this can be identified when the whole month's record is available. Hence at least several months' trial is necessary to establish even an approximate range of variation for any woman.

The relation between the low point on the temperature curve and the characteristic ovulative smear is clear enough. Similarly clear is the relation of the high point with its characteristic premenstrual smear. Although by way of illustration the data on only ten women are presented in this report we have confirmed the existence of the relationship, without exception, in six additional subjects during this fall, thus making a total of sixteen. In establishing this relationship, a convenient indicator has

TABLE 1

The average resting caloric outputs and body temperature of ten women during a single complete menstrual cycle, and showing the differences between the preovulative and premenstrual periods

	ENTIRE MENSTRUAL CYCLE						PREOVULATIVE PERIOD			PREMENSTRUAL PERIOD		
SUBJECT	Number of daily records	Mean Cal./hr.	S.D.	Body temperature	S.D.	Correlation coefficient	Mean Cal./hr.	Body temperature	Correlation coefficient	Mean Cal./hr.	Mean body temperature	Correlation coefficient
1	27	50.3	2.2	98.1	0.4	−0.23	51.8	97.8	+0.44	52.0	98.3	
2	30	59.7	4.1	97.8	0.4	+0.20	60.5	97.7	−0.09	62.8	98.2	+0.38
3	26	51.5	2.9	98.3	0.4	+0.34	48.4	98.0		51.5	98.6	+0.76
4	29	51.3	1.9	98.1	0.3	+0.11	51.1	97.7	+0.56	51.9	98.4	−0.13
5	34	53.9	2.6	97.9	0.4	+0.21	51.3	97.5	+0.06	55.7	98.4	+0.21
6	33	52.6	1.5	98.1	0.4	+0.15	51.6	97.7		52.9	98.5	+0.34
7	32	49.8	2.8	98.4	0.3	+0.09	47.5	98.0	−0.11	50.7	98.6	+0.26
8	26	53.8	2.3	98.0	0.3	−0.08	53.9	97.8	−0.09	56.3	98.5	−0.15
9	29	52.8	2.7	98.0	0.4	−0.10	51.1	97.7	+0.48	56.2	98.5	+0.78
10	29	54.2	2.3	97.8	0.4	+0.16	52.9	97.4	+0.30	54.6	98.2	+0.15

been provided for other changes during the menstrual cycle; temperature-taking is easy and practical. The temperature cycle may therefore be used as a criterion with confidence that it is just as trustworthy as a vaginal smear. None of the common ailments lowers the temperature. Hence pathological deviations are always upward and detectable even if the subject does not provide a history of infection.

Table 1 also presents the basal metabolism data, relating them to body temperatures. Our observations indicate no statistically significant correlation, i.e., basal metabolism, or heat production, varies independently of body temperature. Since body temperature is the sum effect of heat production and heat dissipation, it follows that the body temperature of

the normal subject is dependent primarily upon the activity of heat-dissipating mechanisms. This evidence does not affect the validity of the proposition that "the metabolic rate is dependent on temperatures,—being approximately doubled for every ten degree rise." Within the narrow normal range, the effect of temperature upon metabolism is necessarily small and inextricable from the host of other factors acting simultaneously; while even small changes in metabolic rate would markedly alter body temperature in the absence of compensation by the heat-dissipating mechanisms.

However, the average metabolic rates are consistently slightly lower during the preovulative phase of the cycle than during the premenstrual phase. Since injections of follicular hormone into surgically castrate or into postmenopausal women reproduce the preovulative vaginal smear (13) and also tend to reduce the metabolic rate (14) it seems justified to consider the vaginal smear and the metabolic rate as covariant indices of follicular hormone level. It is also interesting to consider the hypothesis that follicular hormone inhibits not only the gonadotropic function of the anterior pituitary, but also its thyreotropic function: that the basal metabolism is thereby reduced secondarily.

The large day-to-day fluctuations in the metabolic rate of these trained subjects, particularly during the low temperature phase of the ovulative cycle, are also noteworthy; but it is true also of their recumbent blood pressure and pulse rates which, too, are unrelated to the body temperatures. Hence these periods of low body temperature are periods of least homeostasis.

In conclusion, we are happy to express our thanks to Dr. William H. Weir for his preliminary examinations by which the gynecological health of our subjects was ascertained; to Dr. A. J. Carlson, for his advice and interest; to Dr. T. Wingate Todd, Dr. T. T. Zuck and our colleagues of the Associated Foundations whose coöperation has been essential to the favorable prosecution of this work.

SUMMARY

1. There is a constant relation between the lowest body temperatures of the month and the characteristic ovulative smear; and of the highest temperatures with the characteristic premenstrual smear.

2. Basal metabolism is independent of body temperature within the limits of our experiment.

3. There is a similar lack of relation between temperature on the one side and blood pressure or resting pulse rate on the other.

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THE INFLUENCE OF STRENUOUS MUSCULAR EXERCISE UPON RENAL EXCRETION

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Received for publication January 18, 1937

Muscular exercise has long been a favorite subject of investigation by biochemists and physiologists. Many reviews have been published recently which give in detail the work done on the various phases of muscular activty. The present paper presents the results of some observations of the effect of muscular exercise upon renal excretion.

The controversy over the influence of muscular exercise on the composition of urine may be due in part to the difficulty of controlling one or more of the various factors affecting the metabolism and renal function; such as sex, age, health, physical efficiency, state of training, diet, environment, periods of urine collection, and the type, severity and duration of the exertion. One finds descriptions of exercise such as: "The subject ran for one hour until exhausted," "stair running was continued to exhaustion and breathlessness," and "the subjects performed severe muscular exercise for one hour." These statements lack evidence as to the nature, duration and severity of the exertion in terms of the maximum performance for the particular individuals. Bock et al. (1), have shown by blood studies on the Marathon runner DeMar and others that for any given exertion the results of two individuals of different efficiencies do not parallel each other.

While a decrease in urinary excretion of water, total nitrogen and uric acid, following strenuous muscular exercise, has been observed by many workers, creatinine has not been previously reported as showing a marked decrease immediately following exercise. Dunlop et al. (2), and Campbell and Webster (3) noticed an increase in the twenty-four hour excretion of creatinine as a result of muscular exercise. Through the analysis of specimens collected at short intervals following the race Schulz (4) found an increase in creatinine excretion immediately after exercise, which was followed by a decrease after two hours of recovery. Increases in creatinine excretion following muscular exercise have also been reported by many other investigators (5), (6), (7), (8). Contrary to this, Wilson et al. (9) found no significant change in urinary creatinine following one to three minutes of violent stair running. Quick (10) reported the same results

under similar conditions. However, he did observe that a slight increase in excretion followed mild exercise.

EXPERIMENTAL. The experiments in the present investigation were conducted upon two experienced college trackmen who had formerly engaged in long distance track work for a period of several years. Just previous to the investigation they had been training for the 1932 Olympic Marathon. Both were 26 years of age and of equal weight and height. During their college track careers they had been performers of almost equal ability. Thus it is apparent that in many respects they were very evenly matched.

No attempt was made to regulate the diet of the runners. The runs were conducted on the measured circular track at the university stadium and the times and distances of the runs were recorded. Previous records showed that the average maximum performances for the two individuals were as follows: 3 miles in 15 minutes and 30 seconds; 4 miles in 21 minutes; 6 miles in 32 minutes; 10 miles in 56 minutes; and the Marathon distance of 26 miles, 421 yards in 2 hours and 42 minutes.

The urine samples were collected under toluene at relatively frequent intervals. A series of preliminary tests revealed that the subjects were apparently able to evacuate the bladder after short periods of accumulation as indicated by the uniform excretion of creatinine.

Urine samples were taken just before each run and again as soon after the run as possible so that the latter samples represented accumulations of urine over periods, the greater part of which was spent in actual exercise. The time allowed to elapse between the close of the run and the collection of the samples varied from as few as 2.5 minutes in the short runs to 20 to 26 minutes in the long runs. These periods of time were allowed to elapse following the run in order to permit the runner to recover sufficiently to void properly. Immediately upon finishing the run difficulty in micturition was often experienced. This was apparently not due to the oliguria incident to the exertion, but to other factors, since subsequent periods did not necessarily reveal appreciable increases in the rate of excretion.

Methods of determination. The following methods were used for determination: Creatinine, Folin (11); uric acid, Benedict and Franke (12); inorganic phosphate, Youngberg and Youngberg (13); chloride, Volhard-Harvey (14); total nitrogen was determined by micro-Kjeldahl.

DISCUSSION. The data presented in the tables are calculated as relative rate of excretion compared to the "initial rate." The "initial rate" is considered to be the rate of excretion during the period immediately preceding the run. The rate of water excretion was calculated in cubic centimeters per minute, and the rate of excretion for various urinary constituents in milligrams per minute.

The volume of urine excreted was decreased during the period of exer-

cise, as was also the rate of excretion of all urinary constituents. The relative rates of excretion of the various constituents show very little correlation with each other or with the volume of excretion, which makes it improbable that the decreased elimination is due entirely to the oliguria incident upon the exercise.

There is a consistent difference in the effect of exercise upon the rate of excretion of creatinine and of uric acid during and immediately following the run. The recovery to normal rate of excretion after the run was very rapid in the case of creatinine, the recovery being complete and often slightly higher than the initial rate in the first 20 or 30 minute period of excretion immediately following the run. The recovery to a normal rate of uric acid excretion required from 3 to 5 hours. The rate of excretion usually continued to rise to values appreciably above the initial rate of excretion. This difference in the rate of recovery of creatinine and uric acid excretion is also indicated in the percentage of the creatinine nitrogen and uric acid nitrogen of the total nitrogen. The total nitrogen excretion decreases as all renal excretion is decreased during the run. The recovery of the excretion rate of total nitrogen usually lags behind that of creatinine, but is more rapid than that of uric acid, so that within 30 minutes to an hour after the run the percentage of creatinine nitrogen of the total nitrogen has usually increased markedly above the initial value, while the ratio of uric acid nitrogen to the total nitrogen may have decreased below the initial value.

The variation in renal excretion which resulted from altering the intensity of exertion with the same individual may be noted by comparing the tables for subject A on June 15, 16, 17 and 18. On June 15, 16 and 18, the runs were made at approximately six minutes to the mile, which, while not the maximum performance for the man, was sufficiently fast to call forth considerable exertion. On June 17 a slow run of eight minutes to the mile was made which was only a mild form of exercise for the individual. When the runs were made at the rate of six minutes to the mile, there was an average decrease of 77 per cent in the rate of excretion of creatinine during the run, and about 80 per cent decrease for uric acid. The corresponding values on the day of the slow run were less than 2 per cent for creatinine and 35 per cent for uric acid. In all cases there was a very rapid recovery in rate of excretion for creatinine and slow recovery for uric acid. Previous investigators may have overlooked the initial drop in the creatinine excretion partly because of the fleeting nature of the decrease with a rapid recovery, and partly because of a lack of standardization of the exertion in terms of the relative maximum ability and training of the individual. While to run 3 miles in 24 minutes seems to be a considerable amount of work, in the case here reported it was so mild a form of exercise as not to cause a decrease in rate of creatinine excretion.

TABLE 1

The influence of muscular exercise upon the rate of renal excretion of sodium chloride, inorganic phosphate, total nitrogen, creatinine, uric acid and water

June 15, subject A, run 3 miles in 17 minutes, 40 seconds, starting at 5:00 p.m.

TIME OF COLLECTION P.M.	PERCENT OF INITIAL RATE OF EXCRETION						PERCENT OF TOTAL NITROGEN	
	Volume	NaCl	P	Total N	Creatinine	Uric acid	Creatinine N	Uric acid N
4:30– 5:00	100.0	100	100.0	100.0	100.0	100.0	4.9	1.5
5:00– 5:30	60.0	38	44.9	42.0	36.8	27.3	4.8	1.0
5:30– 6:04	41.0	25	108.4	49.5	98.9	53.2	9.8	1.7
6:04– 6:34	36.2	32	83.9	31.6	100.0	78.3	15.6	3.9
6:34– 7:07	31.9	27	83.9	70.0	97.9	91.4	6.9	2.1
7:07– 7:43	29.5	27	70.3	68.6	92.0	93.9	6.5	2.2
7:43– 8:15	34.0	22	101.2	85.6	102.2	97.1	5.8	1.8
8:15– 8:46	36.0	27	101.4	90.0	102.2	126.2	5.6	2.2
8:46– 9:16	44.5	46	110.2	93.6	108.2	144.4	5.6	2.4
9:16–12:10		68	100.0	104.0	105.9	139.3	5.0	2.1

Initial rates of excretion were: Volume, 1.57 ml. per minute; sodium chloride, 13.7 mgm. per minute; phosphorus, 0.55 mgm. per minute; total nitrogen, 8.0 mgm. per minute; creatinine, 1.06 mgm. per minute; uric acid, 0.38 mgm. per minute.

June 16, subject A, runs 3 miles in 17 minutes, 38 seconds, starting at 4:50 p.m.

TIME OF COLLECTION P.M.	PERCENT OF INITIAL RATE OF EXCRETION						PERCENT OF TOTAL NITROGEN	
	Volume	NaCl	P	Total N	Creatinine	Uric acid	Creatinine N	Uric acid N
4:30– 4:50	100	100	100.0	100.0	100.0	100.0	4.5	1.1
4:50– 5:10	20	12	11.6	11.6	12.3	12.7	4.8	1.7
5:10– 5:30	56	18	89.6	32.0	89.9	16.5	12.7	0.8
5:30– 5:50	60	36	91.1	61.1	100.0	33.7	7.4	0.9
5:50– 6:10	66	33	47.9	80.5	88.9	149.5	5.0	2.9
6:10– 6:30	48	31	56.6	81.5	89.9	86.9	5.0	1.7
6:30– 6:50	62	39	46.8	94.1	100.0	71.0	4.9	1.2
6:50– 7:38	50	39	33.8	79.5	94.1	76.0	5.4	1.5
7:38– 8:38	24	28	50.7	64.0	90.9	71.0	6.4	1.8
8:38– 9:38	20	18	53.8	46.6	91.9	67.5	8.9	2.2
9:38–10:38	33	39	54.6	59.2	100.0	120.0	7.6	3.2
10:38– 9:16*	25	28	59.7	61.1	83.3		6.2	

* a.m.

Initial rates of excretion were: Volume, 2.5 ml. per minute; sodium chloride 25.5 mgm. per minute; phosphorus, 0.70 mgm. per minute; total nitrogen, 10.3 mgm. per minute; creatinine, 1.27 mgm. per minute; uric acid, 0.48 mgm. per minute.

TABLE 1—*Continued*

June 17, subject A, run 3 miles in 24 minutes starting at 4:32 p.m.

TIME OF COLLECTION P.M.	PERCENT OF INITIAL RATE OF EXCRETION						PERCENT OF TOTAL NITROGEN	
	Volume	NaCl	P	Total N	Creatinine	Uric acid	Creatinine N	Uric acid N
4:00– 4:30	100	100	100.0	100.0	100.0	100	4.8	1.9
4:30– 5:00	68	24	97.5	73.0	98.2	65	6.6	1.5
5:00– 5:33	66	42	80.5	88.0	98.2	65	5.3	1.3
5:33– 6:03	73	62	72.0	94.0	100.9	78	5.1	1.5
6:03– 6:33	90	78	79.3	107.0	100.0	85	4.5	1.5
6:33– 7:03	60	62	75.3	65.9	98.2	77	7.1	2.2
7:03– 7:33	53	42	105.0	71.7	103.6	83	7.0	2.1
7:33– 8:03	53	39	130.0	82.4	109.1	90	6.4	2.0
8:03– 9:18	69	49	112.0	72.4	111.8	102	7.4	2.6
9:18–10:28	47	39	51.7	78.4	101.8	106	6.2	2.5
10:28–12:33	61	21	70.0	66.3	98.2	75	7.1	2.1
12:33– 8:00*	35	11	140.0	78.4	90.0	63	5.7	1.4

* a.m.

The initial rates of excretion were: Volume, 1.3 ml. per minute; sodium chloride, 23.3 mgm. per minute; phosphorus, 0.41 mgm. per minute; total nitrogen, 8.5 mgm. per minute; creatinine, 1.10 mgm. per minute; uric acid, 0.47 mgm. per minute.

June 18, subject A, run 5 miles in 30 minutes, starting at 10:10 a.m.

TIME OF COLLECTION P.M.	PERCENT OF INITIAL RATE OF EXCRETION						PERCENT OF TOTAL NITROGEN	
	Volume	NaCl	P	Total N	Creatinine	Uric acid	Creatinine N	Uric acid N
8* –10:09	100	100	100.0	100.0	100.0	100.0	7.1	2.4
10:09–10:50	29	53	2.0	37.6	21.0	22.2	3.9	1.4
10:50–11:20	42	51	105.0	41.7	110.2	24.4	19.0	1.4
11:20–11:50	40	64	4.9	85.0	96.5	58.5	8.0	1.7
11:50–12:20	40	67	7.7	95.0	96.5	91.5	7.3	2.4
12:20–12:50	35	41	4.9	91.7	94.6	82.6	7.3	2.2
12:50– 1:20	47	45	11.5	103.0	109.8	108.3	7.3	2.5
1:20– 2:30	44	86	28.0	96.6	110.2	121.7	7.9	3.0
2:30– 3:30	67	145	35.0	125.0	112.0	195.7	6.3	2.9
3:30– 4:30	47	113	50.0	91.7	107.9	113.2	8.3	3.0
4:30– 5:34	40	91	105.0	85.0	90.3	78.0	7.5	2.2
5:34– 6:34	29	91	104.0	83.4	98.2	78.0	8.3	2.3
6:34– 7:34	21	68	197.0	61.6	94.6	72.0	10.8	2.9
7:34– 8:40	30	36	255.0	81.7	107.8	81.0	9.3	2.4
8:40–10:40	28	36	276.0	86.7	107.8	78.0	8.8	2.2
10:40– 9:47*	21	9	465.0	78.3	89.4	50.0	8.0	1.5

* a.m.

The initial rates of excretion were: Volume, 1.4 ml. per minute; sodium chloride, 9.1 mgm. per minute; phosphorus, 0.20 mgm. per minute; total nitrogen, 6.0 mgm. per minute; creatinine, 1.14 mgm. per minute; uric acid, 0.44 mgm. per minute.

TABLE 1—*Continued*

June 21, subject A, run 16 miles in 98 minutes, starting at 2:40 p.m.

TIME OF COLLECTION P.M.	PERCENT OF INITIAL RATE OF EXCRETION						PERCENT OF TOTAL NITROGEN	
	Volume	NaCl	P	Total N	Creatinine	Uric acid	Creatinine N	Uric acid N
12:54– 2:36	100.0	100	100.0	100.0	100.0	100.0	4.8	1.9
2:36– 4:40	9.4	10	48.2	15.5	42.2	7.9	13.1	0.9
4:40– 5:12	17.9	Trace	56.0	35.6	108.2	13.3	14.7	0.7
5:12– 5:42	13.6	Trace	41.8	58.8	102.0	21.3	8.4	0.6
5:42– 6:17	11.7	Trace	53.6	49.9	100.0	16.3	9.7	0.6
6:17– 7:33	22.0	Trace	213.9	71.0	108.2	34.8	7.4	0.9
7:33– 8:33	28.0	5	210.9	68.8	116.3	82.8	8.2	2.2
8:33– 9:33	29.0	10	111.3	105.0	116.3	102.1	5.3	1.8
9:33–10:45	20.0	10	100.9	94.4	104.8	88.1	5.4	1.7
10:45–11:45	21.0	Trace	228.5	68.8	104.8	85.1	7.4	2.3
11:45– 8*	17.4	Trace	482.1	70.0	91.8	36.1	6.3	1.0
8* – 2:36†	21.0	Trace	237.3	46.6	93.2	74.8	9.6	3.0

* a.m.

† p.m.

The initial rates of excretion were: Volume, 2.4 ml. per minute; sodium chloride, 14.8 mgm. per minute; phosphorus, 0.11 mgm. per minute; total nitrogen, 9.0 mgm. per minute; creatinine, 1.17 mgm. per minute; uric acid, 0.50 mgm. per minute.

June 15, subject B, run 3 miles in 17 minutes, 40 seconds, starting at 5:00 p.m.

TIME OF COLLECTION P.M.	PERCENT OF INITIAL RATE OF EXCRETION						PERCENT OF TOTAL NITROGEN	
	Volume	NaCl	P	Total N	Creatinine	Uric acid	Creatinine N	Uric acid N
3:28–4:52	100.0	100	100.0	100.0	100.0	100.0	4.4	1.5
4:52–5:30	66.5	56	84.6	39.0	81.4	47.2	9.2	1.9
5:30–6:02	92.0	43	149.2	64.6	103.8	43.1	7.1	1.1
6:02–6:33	108.0	94	101.8	100.0	110.7	91.5	4.9	1.4
6:33–7:06	104.0	80	97.4	95.0	105.1	95.9	4.9	1.6
7:06–7:44	100.0	64	79.8	79.2	96.6	82.3	5.4	1.6
7:44–8:15	92.0	64	128.3	100.0	95.4	89.2	4.2	1.4
8:15–8:45	92.0	77	110.2	84.1	101.3	100.0	5.3	1.9
8:45–9:17	100.0	64	120.7	74.4	97.7		5.8	

Initial rates of excretion were: Volume, 0.83 ml. per minute; sodium chloride, 12.1 mgm. per minute; phosphorus, 0.63 mgm. per minute; total nitrogen, 8.2 mgm. per minute; creatinine, 0.98 mgm. per minute; uric acid, 0.39 mgm. per minute.

TABLE 1—*Concluded*

June 16, subject B, run 3 miles in 17 minutes, 38 seconds, starting at 4:50 p.m.

TIME OF COLLECTION P.M.	PERCENT OF INITIAL RATE OF EXCRETION						PERCENT OF TOTAL NITROGEN	
	Volume	NaCl	P	Total N	Creatinine	Uric acid	Creatinine N	Uric acid N
4:30–4:50	100	100	100.0	100.0	100.0	100.0	8.5	2.3
4:50–5:10	18	13	16.1	27.0	14.5	8.8	4.6	0.7
5:10–5:30	51	36	25.8	56.1	111.1	27.1	16.9	1.0
5:30–5:50	49	65	108.4	112.0	110.0	59.5	8.4	1.2

The initial rates of excretion were: Volume, 2.3 ml. per minute; sodium chloride, 16.0 mgm. per minute; phosphorus, 0.61 mgm. per minute; total nitrogen, 4.8 mgm. per minute; creatinine, 1.10 mgm. per minute; uric acid, 0.33 mgm. per minute.

June 21, subject B, run 17 miles in 104 minutes, starting at 2:40 p.m.

TIME OF COLLECTION P.M.	PERCENT OF INITIAL RATE OF SECRETION						PERCENT OF TOTAL NITROGEN	
	Volume	NaCl	P	Total N	Creatinine	Uric acid	Creatinine N	Uric acid N
12:54– 2:36	100.0	100	100.0	100.0	100.0	100.0	5.1	1.3
2:36– 4:40	18.2	15	82.2	22.5	83.2	40.8	19.0	2.4
4:40– 5:12	29.0	25	71.3	31.2	117.7	134.4	19.3	5.7
5:12– 5:42	19.0	20	49.0	63.7	105.8	124.1	8.5	2.6
5:42– 6:20	19.0	20	56.9	95.0	97.1	74.4	5.3	1.0
6:20– 7:20	27.0		135.1		116.5	134.4		
7:20– 8:20	34.0		133.7		114.2	134.4		
8:20– 9:33	95.0		168.1		112.1	284.4		
9:33–10:20	92.0		197.9		107.9	130.9		

The initial rates of excretion were: Volume, 2.4 ml. per minute; sodium chloride, 14.8 mgm. per minute; phosphorus, 0.38 mgm. per minute; total nitrogen, 8.0 mgm. per minute; creatinine, 1.11 mgm. per minute; uric acid, 0.32 mgm. per minute.

The effect observed in the influence of severe exercise upon the composition of urine would indicate a certain degree of "damming up" of waste products which is probably due to some renal impairment. Such a condition would account for the rapid recovery in rate of excretion of creatinine, followed by urea (as representing the greater part of the total nitrogen determined) and finally by uric acid. Blood studies support this view. Rakestraw (15), (16), and Levine et al. (17), found increases in the non-protein nitrogen and uric acid in the blood during exercise. Feigl (18) and Kael (19) noticed increases in blood creatinine during and immediately following exercise.

In the present experiments the rate of excretion of inorganic phosphates was decreased during the run and the recovery followed somewhat that of uric acid. The "sudden fleeting" increase a short time after severe exercise which was reported by Havard and Reay (20), was confirmed.

The rate of chloride excretion was decreased during and after the run. Chloride is essential in maintaining water balance in the tissues and also an appreciable amount of chloride is lost in perspiration. The rate of excretion of chloride may remain low for a considerable period of time after the run. This is shown especially in the 16 mile run of subject A on June 21, when twenty-four hours later the traces of chloride being excreted were too small to be determined by the Volhard-Harvey method which was used. The excretion of chloride differs from the excretion of the other constituents reported in this paper in that it is a threshold substance.

Differences may be noted between subjects A and B in the results reported for the long run of June 21. There was also a difference in the performance of the two runners. Subject A failed suddenly, ceased sweating, and was forced to discontinue the run at the 16th mile, whereas subject B finished the 17 miles in good condition. Subject A exhibited an oliguria following the run which lasted for many hours despite the intake of large amounts of water.

The experimental data of this paper indicate that the depression of renal excretion involves actual change in the functional capacity of renal tissue. Additional evidence in support of this view is afforded by the work of Benzinger (21), who found that the Van Slyke kidney function test revealed a 25 to 75 per cent decrease in kidney function. Van Slyke et al. (22) also found instances in which kidney function was depressed to 35 and 44 per cent of normal as indicated by urea clearance tests.

The albumin findings of Barach (23) also support the view that changes in the function of renal tissue occur. His observation that albuminuria may persist for three weeks or more following a particularly hard race indicates that the disturbance in these cases is not only temporary, but involves renal changes of a more permanent nature.

CONCLUSIONS

1. During and following muscular exercise there is a marked decrease in the excretion of creatinine as well as the other urinary constituents, total nitrogen, uric acid, chlorides and inorganic phosphates.

2. The depression of urinary excretion following exercise is influenced by the severity of the exertion relative to the maximum ability and training of the individual.

3. The extent and duration of the period of depression of excretion of the various urinary constituents varies for each individual substance and is not dependent upon the rate of water excretion.

4. The recovery from the condition of decreased rate of excretion is much more rapid in the case of creatinine than of uric acid.

5. The results of the experiments presented indicate that the decreased rate of excretion during and immediately following severe exercise is due to renal impairment.

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THE EFFECTS OF SODIUM DEPRIVATION ON THE ANIMAL ORGANISM

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Received for publication January 19, 1937

Numerous investigations on the mineral requirements of the animal organism have emphasized the necessity of an adequate supply of salts in the diet. Thus far only certain effects of specific mineral deficiencies have been investigated. On the basis of their classic work in 1918, Osborne and Mendel (1) concluded that the "law of the minimum" holds for all essential salts in the diet, failure of growth in the whole body resulting where the limiting factors are deficiencies in the salts of chlorine, sodium, magnesium, potassium, calcium and phosphorus. These investigators found that rats grew normally on a ration containing 0.035 per cent sodium. Mitchell and Carman (2), Miller (3), St. John (4), Schoorl (5) and Sjollema (6) reported a decreased rate of growth when the sodium content in the food is low. In addition, Miller (3) observed sterility of the female rat on this type of diet. St. John (4) demonstrated further effects of sodium deficiency. On such diets reproduction was abnormal and the eyes of many of the rats were seriously affected, blindness resulting in many cases. Although the above-mentioned experiments have elucidated to some extent the importance of sodium, the diets contained considerable amounts of sodium and, almost without exception, they were obviously deficient in some other dietary essentials. In this report it will be shown that these difficulties have been largely overcome. The sodium content of the experimental diet has been reduced to 0.002 per cent and other nutritive essentials have been provided in adequate amounts as demonstrated by the growth rate of rats restricted to this diet supplemented with sodium bicarbonate. This marked reduction in dietary sodium has been effected by the use of a new method (7) of extracting the vitamin B complex from yeast.

EXPERIMENTAL. A total of ninety experimental and fifty control rats were used in this investigation. Weaned rats weighing 35 to 40 grams were placed on the diets, the composition of which appears on the following page.

Sodium-low Diet

Acid washed, alcohol extracted casein	18.0
Methyl alcohol-gaseous HCl yeast extract = to dry yeast	5.0
Salt mixture no. 18a	5.3
Commercial sucrose	66.7
Sweet butter fat	5.0
Viosterol, 15 drops per kilo	

Control Diet

Acid washed, alcohol-extracted casein	18.0
Salt mixture no. 19	6.7
Methyl alcohol-gaseous HCl yeast extract = to dry yeast	5.0
Commercial sucrose	65.3
Sweet butter fat	5.0
Viosterol, 15 drops per kilo	

Salt Mixture no. 18a

$CaCO_3$	2.0
KCl	1.0
MgO	0.2
KH_2PO_4	1.5
Ferric citrate	0.5
$CuSO_4 \cdot 5\ H_2O$	0.1
	5.3

Salt Mixture no. 19

$NaHCO_3$	1.4
$CaCO_3$	2.0
KCl	1.0
MgO	0.2
KH_2PO_4	1.5
Ferric citrate	0.5
$CuSO_4 \cdot 5\ H_2O$	0.1
	6.7

In preparing the methyl alcohol-gaseous HCl extract, in order to be sure of obtaining an extract of the activity described in the original paper (7) it is of utmost importance to have the proper hydrogen-ion concentration and, furthermore, after completion of the methyl alcohol-gaseous HCl treatment, the acidified mixture should be heated to boiling and filtered. The residue is washed with absolute methyl alcohol. The filtrate and washings are then allowed to stand in the cold overnight. If these precautions are not observed the extraction of the vitamin is incomplete.

At about this time crystalline B_1 and lactoflavin had become available. It seemed worthwhile, therefore, to investigate the effect of the same sodium-low diet substituting as the source of the vitamin B complex, Merck's crystalline vitamin B_1 (20 γ per rat per day), lactoflavin (40 γ per rat twice weekly), and vitamin B_6 in the form of Peter's eluate (1 cc. per rat per day) (8). Twelve rats on the experimental and six on the control diet were used for this purpose. The symptomatology produced was similar to that when the methyl alcohol-gaseous-HCl extract was used as the source of the vitamin B complex in the ration. The methods for preparing the casein and the diet, and the care of the animals are the same as those described in a previous publication (9).

The animals on the sodium-low diet ate well and their food intake was normal as compared with the control rats. The experimental animals grew fairly rapidly for a few weeks and then began to lose weight (chart I). No noticeable symptoms were observed until about the sixth to eighth week, when the difference in appearance of the experimental and control animals was more striking than the difference in growth. At this time the experimental animals began to show eye changes. Although some of the

rats had normal corneae at this time, others began to exhibit a bluish-grey cornea; some had perforated ulcers and all showed a thin sanguinolent secretion covering both eyes (Table I).

During the next two weeks, practically all experimental animals developed the following progressive eye changes: thickening and bluish-grey appearance of the corneae, sanguinolent secretion, corneal ulceration, perforation of the ulcers, hypopyon, marked bulbar and ciliary injection, edema of the lids, loss of hair of lids, loss of lashes and abscess of the anterior segment of the eyes.

At this point, the eighth week of the experiment, nineteen rats were selected for histological studies of the eye. They were divided into two groups, A and B. The animals of group A were killed, their eyes were re-

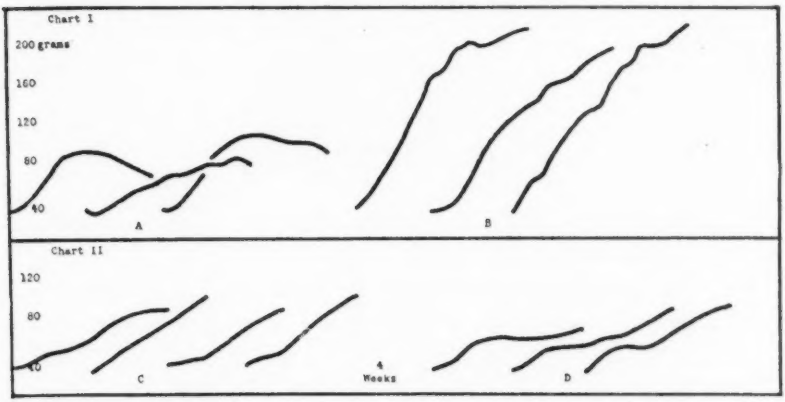


Chart I. Group A represents the growth of animals fed the sodium-low diet. Group B represents the growth of animals fed the control diet containing sodium.

Chart II. Group C represents the growth of animals fed the chlorine-low diet. Group D represents the growth of animals fed the sodium and chlorine-low diet.

moved and prepared for histological examination by fixing in Bouin solution, imbedding in paraffin, sectioning and staining in hematoxylin and eosin. Those of group B were continued on the experimental diet. These animals died in the twentieth week of the experiment, their eyes showing perforated ulcers and abscess of the anterior segment of the eye. Microscopic studies of the eyes of this group were also made.

The histological changes of the eyes of the rats eight weeks on the sodium-deficient diet consisted in some cases of corneal lesions with extensive anterior synechiae, also posterior synechiae and beginning capsular cataract. Wandering cells were present in the posterior chamber. In others, the corneal epithelium was thickened in places and showed areas of ulceration. The whole thickness of the cornea was infiltrated with wan-

dering cells of all types and there was apparently perforation, for the iris was adherent to the center of the cornea in this region. There were some round cells on the surface of the iris in the posterior chamber. The lacrimal gland appeared to be normal.

The sections of the eyes of the rats twenty weeks on the experimental diet showed the following changes: in a number, there was intense infiltration throughout the cornea, which had evidently been associated with per-

TABLE 1

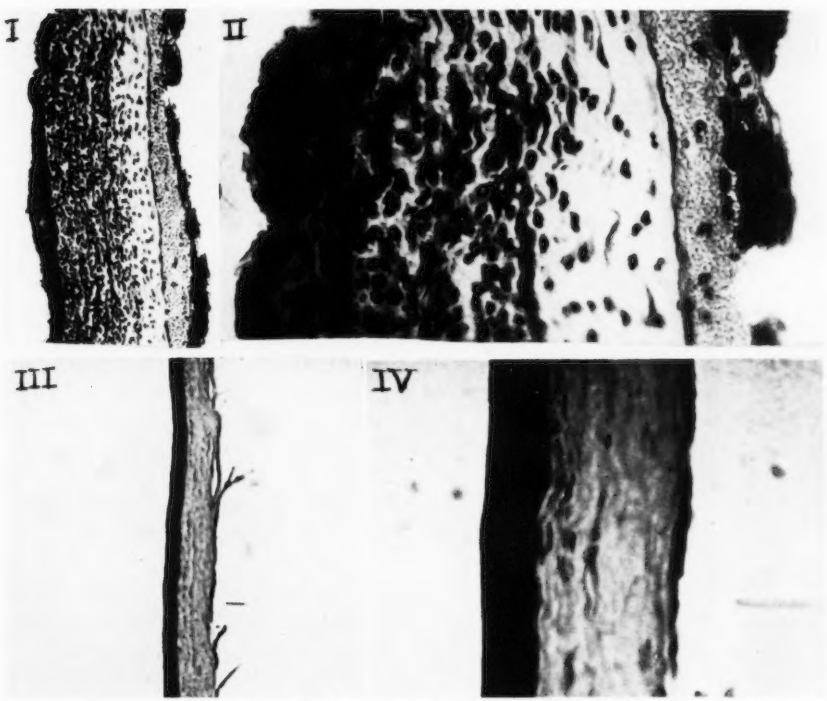
Gross eye lesions in living animals during 6th, 7th and 8th weeks of the experiment

	RAT NUMBER	LIDS	HEMORRHAGIC SECRETION ON LIDS AT INNER CANTHUS	LACRIMATION	CORNEA	CILIARY AND CONJUNCTIVAL APPEARANCE	ULCER	HYPOPYON
6th week	1	Normal	Present	Slight increase	Clear			
	2	Normal	Present	Slight increase	Clear			
	3	Edema	Present	Normal	Bluish-white opaque			
	4	Marked edema	Present	Normal	Grey, opaque		Present and keratectasia	
	5	Marked edema	Present	Normal	Grey, opaque		Present	Present
7th week	1	Edema	Present	Normal	Bluish-grey			
	2	Slight edema	Present	Normal	Left-clear right-opaque			
	3	Edema	Present	Normal	Left-grey and thickened		Left eye only present	Left eye only present
	4	Marked edema	Present	Normal	Grey	Marked congestion	Perforated	Present
	5	Marked edema	Present	Normal	Grey	Marked congestion	Perforated	Present
8th week	1	Marked edema	Present	Normal	Grey	Left eye congested	Left eye only present	Left eye only present
	2	Marked edema	Present	Normal	Grey, opaque	Marked congestion	Present	
	3	Marked edema	Present	Normal	Opaque		Present	Present
	4	Marked edema	Present	Normal	Grey	Marked congestion	Present	Present
	5	Marked edema	Present	Normal	Grey	Marked congestion	Present	Present

foration of an ulcer, inasmuch as the iris was adherent to the back of the cornea over its whole extent. The corneal epithelium is composed of thin, basal cells which are flattened. The superficial two-fifths are keratinized; in certain animals there was intense purulent keratitis with perforated ulcer and hemorrhage into the anterior chamber. In places, the corneal epithelium was greatly thickened. Basal cells were irregular in pattern, the superficial layer was keratinized. There was a thick plaque of hyaline material over the ulcer which contained colonies of bacteria.

The sections of the eyes of the control animals were all normal.

Since keratinization of the corneal epithelium was found in the sodium-depleted rats and since keratinization of epithelial structures is characteristic of vitamin A deficiency, the histopathology of the eyes of the sodium-deficient animals was studied and compared with that of the vitamin A-deficient animal. As is noted elsewhere in this paper, the diet contained



Photomicrograph of section of eye of rat 20 weeks on the sodium-low diet. Figure I, 40; figure II, ×150.

Note the greatly thickened corneal epithelium, intense purulent keratitis and hemorrhage into the anterior chamber.

Photomicrograph of section of eye of rat 20 weeks on the control diet. Figure III, ×40; figure IV, ×156.

sufficient amounts of vitamin A. Various investigators agree that in vitamin A deficiency there is diminished lacrimation and dryness of the eyes. In this experiment, however, the eyes in every case appeared to be bathed in a normal amount of tears (table 1), and in some instances lacrimation increased. Furthermore, Wolbach and Howe (10) state that in vitamin A deficiency they "have not a single example of ulceration of the cornea or of

hypopyon" which is in marked contrast to the findings in this study. It is obvious that the eye disturbances of the rat occurring as a result of sodium deprivation are markedly different from the changes in the eye produced by vitamin A deficiency.

As the eye condition progressed, the rats rapidly declined in weight, many of them dying. There was 100 per cent mortality after 18 to 21 weeks on the experimental diet. Postmortem examinations were made of all the animals. The body was lean; the adipose tissue when present was very sparse. There was marked atrophy of muscle tissue. The lungs were badly infected. The liver was a darker reddish brown than normal and was also mottled. The spleen was very dark and greatly reduced in size. The kidneys were darker and occasionally showed mottling. The suprarenals were orange in color as contrasted with the pale pink of the controls. Frequently the bladder was distended, and the stomach and intestine were filled with gas. Abscesses or cysts were observed on the small lymph glands situated in front of the submaxillary glands in a large number of the rats.

The bones were fragile, softer and cut more easily than the controls. When stained with silver nitrate, the femurs and tibiae appeared to be slightly rarified and osteoporotic, which phenomena were not exhibited by the control animals. Microscopic examination of the tibiae of the rats showed very little cartilage and little osteoid tissue. The cartilage band was narrow and flat. The picture of these bones is very similar to that seen in hypothyroid animals.

The testes of rats dying between the 18th and 20th week on the diet were smaller than those of the controls. The epididymides of these animals appeared to be atrophied.

Reproduction. A series of experiments to investigate the effect of sodium deprivation on reproduction was set up. First, the oestrous behavior of rats on the experimental diet was studied. Maturity was somewhat delayed, occurring on the average about the 66th day of life in contrast to the 58th day of life of the control rats. Vaginal smears were made following the technic of Long and Evans (11). A group of twelve rats on the experimental diet, and another group of eight on the control one, were used for this purpose. After establishment of the vaginal canal, smears were taken daily for a period of 56 days. The control animals showed no abnormality in the vaginal smears. These rats exhibited an oestrous cycle of approximately $4\frac{1}{2}$ days, which is within the normal range. The ovulation rhythm of the sodium-depleted rats was gravely affected. The experimental rats behaved as follows during this period: for an average of 14 days the ovulatory performance of the sodium-low rats was approximately normal. Thereafter a characteristic disturbance was observed in

the oestrus of the depleted animals resembling that of vitamin A deficiency. It consisted in prolongation of the oestrous desquamation stage in the vaginal epithelium, the smears showing almost exclusively large, flaky, cornified cells which normally characterize the actual period of oestrus and ovulation, but which in this case occurred throughout the entire depletion period. In addition to the cornified cells, there were present small amounts of mucus; in some cases, excessive numbers of leucocytes; and in others, a few cornified, nucleated epithelial cells were seen. This abnormality in the vaginal smears of the depleted rats appeared about the same time as the initial changes in the eyes but preceded the growth decline.

In spite of the fact that the diet contained butter fat in amount known to be sufficient, due to the similarity in the vaginal picture to that of the smear in vitamin-A depleted rats, it seemed advisable to rule out the possibility of a borderline vitamin A deficiency resulting from impaired utilization of this vitamin. Therefore, both butter fat and carotene respectively were administered orally. However, repair of the vaginal epithelium was not demonstrated following vitamin A therapy although these substances were administered in large doses.

In order to further determine the effect of sodium depletion on the reproductive systems of both males and females, mating experiments were carried on with rats maintained on the sodium-low diet from the time of weaning and with rats from the stock colony whose reproductive histories were known. During a period of about ten weeks following the attainment of sexual maturity, sodium-depleted females were mated with males from the stock breeding colony. The rats were housed in individual cages. Each female was introduced into the cage of the stock male for a period of 10 to 12 hours out of each 24 hours. During this time the rats had no access to food. During the remainder of the 24 hours both the stock males and the sodium-depleted females were returned to their individual cages where they were fed their respective diets.

Although these females were under observation during the day throughout the experimental period, copulation was not observed. However, from about the 13th day of the experiment there was a slight increase in weight which was suggestive of pregnancy and which was maintained throughout the remainder of the survival period. Postmortem examination at the end of the ten week period revealed no signs of pregnancy in ten of the sodium-depleted animals. The remaining two females, nos. 73 and 81, behaved somewhat differently. They gained weight slowly but to a greater extent than the other sodium-low rats. During a period of 43 days, rat 73 gained 46 grams and rat 81, 49 grams. At that time they were sacrificed. In rat 73 but one fetus was found. Rat 81 had five fetuses which were all located in one uterine horn. These fetuses appeared

to be normal except in size. The one in rat 73 weighed 3.6 grams. Of those in rat 81, one weighed 4.5 grams, another 2.2 grams, and the total weight of the remaining three was 1.7 grams. If these findings are the result of sodium depletion, it may indicate that sodium deficiency causes a prolongation of the gestation period.

In order to study the effect of sodium depletion on fertility in the male rat, twelve males were selected which had been restricted to the experimental diet for a period of 6 weeks after weaning. The sperm motility test, gross examination of the testes at autopsy and mating tests with female rats from the stock breeding colony whose fertility records were known, were the tests used for this purpose. Testicular smears were made at 2 week intervals starting with the 40th day on the experimental diet and were continued to the 110th day. The motility continued to be normal till about the 96th day on the diet, when in five of the animals studied there was a decrease in motility. Simultaneously the epididymides appeared somewhat atrophied and there seemed to be a decrease in their fluid content. Although the testes were smaller than those of the controls, they were normal in proportion to the body weight. No abnormality was noted in their appearance.

Six other males were used for the mating experiments. The procedure for mating, housing, feeding, and general care was similar to that followed in mating the sodium-depleted females with stock males. Vigorous females which had been reared on the stock diet and were known to be fertile were introduced into the individual males' cages. Vaginal smears were run on these females in order to observe the mating behavior. Frequent matings were made for a period of about 8 weeks. In this case the females became impregnated. Two females delivered first litters 22 days, another 27 days, after mating. The litters were normal in size, varying from 9 to 11 young per litter. The young were normal in weight and appearance. These experiments were discontinued after each female delivered two litters, which was before the time when the testicular smears of males on the diet began to show signs of degeneration. In addition to motility, these observations prove that the sodium-depleted male remains fertile for at least 75 to 80 days after restriction to the sodium-deficient diet.

EFFECT OF DIETS DEFICIENT IN CHLORINE AND SODIUM CHLORIDE. It seemed to be of importance in connection with this investigation to observe the effect of the deprivation of chlorine alone, and of both sodium and chlorine. The symptomatology produced by these rations was compared with that caused by the sodium-low diet. The composition of these diets was the same as the one deficient in sodium only with the exception that the formulae for the salt mixtures were as follows:

Cl-free		*NaCl-free*	
$CaCO_3$	1.5	$CaCO_3$	1.5
$K_2CO_3 \cdot 1\frac{1}{2}\ H_2O$	1.1	$K_2CO_3 \cdot 1\frac{1}{2}\ H_2O$	1.1
KH_2PO_4	1.7	KH_2PO_4	1.7
MgO	0.2	MgO	0.2
$NaHCO_3$	1.7	Fe citrate	0.5
Fe citrate	0.5	$CuSO_4 \cdot 5\ H_2O$	0.1
$CuSO_4 \cdot 5\ H_2O$	0.1	Per 100 grams	5.1
Per 100 grams	6.8		

Eight rats were placed on each of these diets. In both cases growth was retarded (chart II). The animals on the chlorine-low diet exhibited no other symptoms although the experiments were carried on for 90 days. The rats on the sodium and chlorine-deficient diet grew more slowly than the chlorine-depleted animals. However, they were not as retarded in growth as the sodium-depleted rats. After the third week on the diet there were pronounced denuded areas about the shoulders and back. At this time the animals were very apprehensive. At the end of the first month, the mouth, ears, nose and forepaws were bloody; the urine was very dark brown in color. In the fifth week the loss of hair became more marked and extended over larger areas. This increased in intensity through the experimental period. However, it seemed to be restricted to the anterio-dorsal parts of the body only. In relation to the effects of sodium deprivation it is of special interest that during the entire experimental period of 90 days, the eyes of these rats were in normal condition.

On postmortem examination the thymus and liver indicated possible internal hemorrhage. The testes appeared too small for the size of the animal, but otherwise seemed to be in good condition as far as could be judged by gross examination. The adrenals were noticeably different from the controls; they were smaller in every case. The bone cut hard as in the controls, whereas the bone of the sodium-depleted rats cut very easily.

Discussion. The results of the effect of sodium deprivation on the animal organism present several points of interest. The symptomatology as demonstrated by the staphyloma of the eyes, lymph gland abscesses and lung infection indicates that the sodium-depleted animals have a decreased resistance to infection, which condition may not be due directly to sodium depletion, but may be rather an indirect effect of the deficiency. Apparently the experimental animals die of an upper respiratory infection.

The retardation in growth cannot be attributed to a decrease in the food intake since the food consumption of the experimental and control animals was practically the same. This fact suggests that the decrease in growth may be the result of a disturbance in the metabolism of the animal.

Perhaps the outstanding symptoms were the effect of sodium depletion on the adrenals and eyes of rats. It is well known that the aqueous humor maintains the nutritive state of the lens. The composition of the inorganic constituents of the aqueous humor is essentially the same as that of the blood, the Na-Ca-K balance being the same in the lens as in the blood. In the diseased lens this salt balance probably is disturbed. Since the lens is extremely sensitive to salt balance, and to changes of osmotic pressure, it is possible that the abnormal condition of the eyes may be attributed to a disturbance of the sodium-calcium-potassium balance. A second possibility is that resistance to infection of these animals is decreased and may be the cause of the disturbance observed.

The preliminary microscopic findings of the bones of sodium-depleted animals are similar to those of bones of other animals which are retarded in growth. As microscopic examination showed, there was little cartilage in the tibiae of the depleted rats. Since normally cartilage is especially rich in sodium, the withdrawal of it from the organism apparently produces the marked effect observed. The tentative assumption is made that the effect on the bone is not a specific one of sodium deficiency but is due to a general slowing down of growth.

In evaluating the results of the experiments on reproduction the fact is emphasized that the mineral content of the diet is of great importance in reproduction as well as growth. The studies presented in this paper demonstrated that sodium depletion gives rise to serious disturbances of reproductive function. The sodium-depleted male rat is fertile for at least 75 to 96 days after restriction to the experimental diet as is proven by sperm motility and mating tests. In the case of the female, however, the changes produced by the deprivation of sodium appear to be entirely different in etiology and nature from the much simpler disturbance characteristic of vitamin A or vitamin E deficiency.

It is evident that sodium depletion may postpone the attainment of sexual maturity of the rat and that this deficiency is inimical not only to growth but to the normal rhythm of ovulation. Since the continuance of oestrous rhythm in the ovary and vaginal epithelium represents cell activity and growth, it may be assumed that sodium deprivation provokes an abnormal physiology of the reproductive system. The mating experiments apparently indicate that reproduction on the sodium-deficient diet is impossible after a certain time due to sterility of the female. These findings support Miller's and St. John's observations on the effect of sodium-low diets on reproduction. The results obtained in the two instances where impregnation did occur are difficult to interpret. However, since ovulation appears to be abnormal it may be deduced that the reproductive processes of the female rat are affected by sodium depletion.

SUMMARY

Restriction of rats to a diet containing only 0.002 per cent sodium but adequate amounts of other nutritive essentials produces a series of symptoms which is terminated by death. The symptomatology comprises retarded growth, disturbances of the eyes and reproductive function.

Extensive corneal lesions, ulceration, hypopyon, hemorrhage and bulbar and ciliary injection are observed in the sodium-depleted rats. Keratinization is seen in the cornea but the lacrimal gland is normal.

The sodium-depleted male remains fertile for at least 75 to 96 days after restriction to the sodium-deficient diet.

In the female on this diet sexual maturity is delayed, the oestrous rhythm and reproductive processes are affected.

In mating the sodium-depleted females with stock males, copulation is not observed. In most cases no signs of pregnancy are exhibited.

It is shown that the disturbances in the eyes and oestrus occurring in the sodium-depleted rats are different from similar disturbances produced by vitamin A deficiency.

Postmortem examination shows that practically all the tissues are affected. The change in the color of the adrenals is especially noteworthy. The bones of these rats are soft and easy to cut.

A chlorine-low diet and a sodium and chlorine-low diet respectively lead to a symptomatology considerably different from that exhibited by the sodium-low diet. Growth is retarded in both cases, but not to the extent of that in sodium deprivation alone. On the sodium and chlorine-low diet alopecia is observed, which is restricted to the anterio-dorsal part of the body. The eyes and bones of the rats on these diets are normal.

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